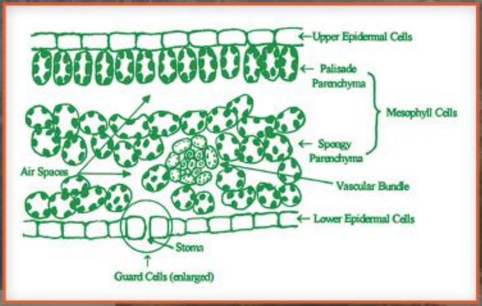
Third Edition

ENVIRONMENTAL TOXICOLOGY

Biological and Health Effects of Pollutants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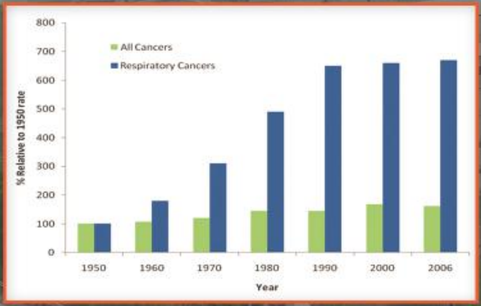

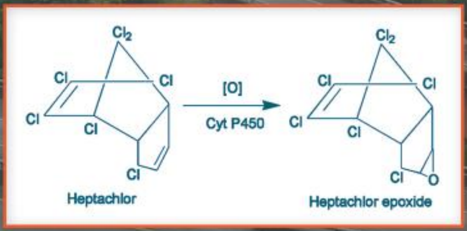
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Ming-Ho Yu
Humio Tsunoda
Masashi Tsunoda

CRC Press
Taylor & Francis Group

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Humio Tsunoda
Masashi Tsunoda

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Preface to the Third Edition

Many changes have occurred since the second edition of this book was published. Some of the changes include further global warming, growing world population, advancing technology and world economy, and expanding industrialization. Yet other changes include worsening air and water pollution, acid rain, and depletion of the ozone layer. In this volume, the information covered previously is updated or expanded.

I welcome Professor Humio Tsunoda and Dr. Masashi Tsunoda as coauthors. Together, they contributed a new chapter, "Occupational Toxicology" (Chapter 14). I am convinced that the material covered in the chapter enriches the content of this volume.

This book is written primarily as an introductory textbook for upper-level undergraduate and beginning graduate students majoring in environmental science, environmental toxicology, environmental health, and related fields. It is hoped that students as well as professionals interested in knowledge concerning the health and biological impacts of pollutants on living systems will find this volume a useful text or source book.

To assist with the students' studies, review questions are placed at the end of each chapter. A *Solution Manual* has also been prepared separately.

Acknowledgments

I wish to express my sincere appreciation to my former advisers and mentors: the late Professor Ho Fang-Kai at National Taiwan University and Professor Gene W. Miller and the late Professor D.K. Salunkhe at Utah State University. Their guidance and kind help have contributed much to my teaching and research career. I am indebted to my wife, Ervena, for her support and encouragement, and I thank my three children, Albert, Christina, and Charlie, for their technical assistance. I thank Joseph Clements and his associates at CRC Press/Taylor & Francis Group for their patience and assistance.

Ming-Ho Yu

About the Authors

Dr. Ming-Ho Yu is professor emeritus at Huxley College of the Environment, Western Washington University. He taught environmental toxicology and environmental health at the university from 1970 to 1997. He received his BS from National Taiwan University in Taipei, Taiwan, and MS and PhD from Utah State University in Logan, Utah. He undertook postdoctoral study at Utah State University and the University of Alberta in Canada. While teaching at Western Washington University, Dr. Yu took a year of sabbatical leave and pursued research as a visiting professor at the Department of Hygiene and Public Health at Iwate Medical University in Japan. He also spent a summer as visiting professor to conduct research at the Institute of Whole Body Metabolism in Chiba, Japan.

Dr. Yu served as the vice president and president of the International Society for Fluoride Research (ISFR) from 1986 to 1996 and is the associate editor of *Fluoride*, the official publication of the society. He is a founding coeditor of *Environmental Sciences*, a journal published in Tokyo, Japan. He was also coeditor of *Environmental Fluoride 1985*, published by Elsevier Science in 1986. Dr. Yu is the author of *Environmental Toxicology—Impacts of Environmental Toxicants on Living Systems* and *Environmental Toxicology, 2nd Edition, Biological and Health Effects of Pollutants* and is coauthor of *Introduction to Environmental Toxicology,* editions 1–4, published by CRC Press.

Dr. Humio Tsunoda, professor emeritus at Iwate Medical University in Morioka, Japan, received his PhD from Hokkaido University in Japan. He received his MD by passing a national examination. He was a professor and chair in the Department of Hygiene and Public Health at Iwate Medical University for 27 years. Between 1997 and 2007, Professor Tsunoda served as a member of the Science Council of Japan, specializing in the area of environmental health. He received a Green Cross Award from the Japan Industrial Safety and Health Association, and awards for distinguished service from the Ministry of Construction; the Ministry of Labor; the Japanese Association of Rural Medicine; Japan Society for Atmospheric Environment; and Japanese Society for Occupational Health. In 2010, Professor Tsunoda received the Order of the Sacred Treasure, Gold Rays with Neck Ribbon from the government of Japan.

Dr. Masashi Tsunoda is an associate professor at the Department of Preventive Medicine and Public Health, Kitasato University School of Medicine in Japan. He received a PhD in social medicine from Niigata University in Japan, an MPH from the University of Pittsburgh, and a PhD in toxicology from the University of Georgia. He is a councilor of the Japan Society for Biomedical Research on Trace Elements; an editorial board member of the International Society for Fluoride Research; and a board member of the Japanese Society of Immunotoxicology. He is coeditor of *Kitasato Medical Journal* and a guest reviewer of *Toxicological Sciences*.

1 Introduction

1.1 STUDY OF ENVIRONMENTAL TOXICOLOGY

Environmental toxicology is concerned with the effects of environmental toxicants on the health of living organisms and the environment. Environmental toxicants are agents released into the general environment that can cause adverse effects on health. The study of environmental toxicology stems from the recognition that (a) human survival depends on the availability of clean air, water, and food and on the welfare of plants and animals; and (b) anthropogenic chemicals as well as naturally occurring chemicals can cause adverse effects on living organisms and ecological processes. The study of environmental toxicology thus focuses on how environmental toxicants, through their interaction with humans, animals, and plants, influence the health and welfare of these organisms.

1.2 IMPORTANCE OF ENVIRONMENTAL TOXICOLOGY AS AN AREA OF SCIENCE

Environmental toxicology is a multidisciplinary science that encompasses several diverse areas of study. Related areas include biology; chemistry (inorganic, organic, and analytical chemistry and biochemistry); anatomy; genetics; physiology; microbiology; ecology; soil, water, and atmospheric sciences; public health; epidemiology; statistics; and law. Compared with many other fields of study, environmental toxicology is a relatively young branch of science. However, its importance has been widely recognized. Indeed, it is one of the most rapidly growing branches of study. A growing number of colleges and universities across the United States and Canada have been offering the course and related programs. A similar trend is seen in a growing number of institutions in other parts of the world.

Obviously, a large number of scientists in the United States and various other countries are pursuing careers directly or indirectly associated with environmental toxicology. The importance of their contributions to the enhancement of environmental quality and human welfare has become increasingly recognized.

1.3 INTRODUCTION TO THIS BOOK

This book provides fundamental information concerning the effects of environmental toxicants on living systems. The book consists of 17 chapters. Appendix 1 presents information on ecological risk assessment.

This chapter discusses the purpose of the study of environmental toxicology. It stresses that its study stems from the recognition that human survival depends on the availability of clean air, water, and food; the welfare of plants and animals; and the environment, and that naturally occurring as well as anthropogenic toxicants can cause a variety of adverse effects on the health of living organisms and ecological processes. This is followed by pointing out the importance of the study of environmental toxicology as an area of study that is a rapidly growing in the United States, Canada, and other countries in the world.

Chapter 2, "Environmental Changes and Health," reviews the dramatic environmental changes that have occurred in recent decades and discusses how these changes have impacted the environment and living systems. Environmental changes discussed include world population; climate changes, particularly global warming; rising seawater acidity; air pollution; water pollution; and soil pollution and how these changes have an impact on plants, animals, and humans. A special emphasis is placed on changing human diseases such as cancer, birth defects and child mortality, reproductive damages, respiratory diseases, endocrine disruption, and diseases induced by metals.

The sources of environmental toxicants and the way in which they are produced are reviewed in Chapter 3, "Occurrence of Toxicants." The chapter also provides a brief review of several major pollution episodes or disasters that occurred in recent decades in the world.

The general manner in which environmental pollutants exert their toxic actions on plants, animals, and humans is discussed in Chapter 4, "Toxic Action of Pollutants." For plants, the sources, uptake, transport of pollutants, and the resultant injury are presented. For mammalian organisms, exposure to pollutants, uptake, transport, storage, metabolism, excretion, and mechanism of toxic actions are covered. Detailed examples are given explaining the mechanism of the toxic action of pollutants. These include disruption or destruction of cellular structure, combination with cellular constituents, and inhibition of enzyme activities. For the last aspect, inactivation of cofactors, binding to the active site of the enzyme itself, and inactivation by toxic metabolites through chelation and metal shift are reviewed. The discussion also includes free-radical reactions and a basic concept on endocrine disruption.

Many factors can influence the toxicity of xenobiotics. In Chapter 5, "Factors Affecting Xenobiotic Action," some of the general factors are addressed. For example, physicochemical properties of toxicants; concentration, mode, and duration of exposure; environmental factors; interaction among different toxicants to produce additive, potentiating, synergistic, or antagonistic effects; and a variety of biological factors are presented. In addition, nutritional factors are discussed, including a review of such nutrients as carbohydrates, protein, lipids, minerals, and vitamins such as vitamins A, D, E, and C.

"Biotransformation: Metabolism of Xenobiotics" is presented in Chapter 6. It reviews the process whereby xenobiotics are converted in the body and the sum of all chemical reactions that occur within a living cell. The discussion begins with the two phases of biotransformation, phase I and phase II reactions, which include oxidation, reduction, hydrolysis, and conjugation. In addition to the mechanism of biotransformation, its characteristics, consequence, and the factors that affect the reaction are addressed. The chapter closes with the discussion of cytochrome P450s.

As is clear, living organisms are exposed to a variety of environmental toxicants. On the other hand, living organisms often possess certain defense mechanisms that enable them to defend against the actions of those toxicants. In addition, they take in various essential nutrients from their diet and the immediate environment. Examples are given in Chapter 7, "Responses to Environmental Toxicants," showing how plants, animals, and humans are equipped with such defense mechanisms. For example, in plants a kind of polypeptide called *phytochelatin* is produced in the cell that is capable of alleviating the toxic effect of heavy metals such as cadmium (Cd) and lead (Pb). Different endogenous antioxidants, such as vitamins C and E and glutathione, are also present in cells, together with antioxidant enzymes such as superoxide dismutase (SOD), catalase, gluthione peroxidase, and glutathione reductase. All these can help counteract the toxic effect of free radicals in plant cells.

In humans and animals, the respiratory tract, membranes, liver, and kidneys are equipped with mechanisms that can counteract the toxic effects of various toxicants. For example, the liver plays a foremost role in detoxifying xenobiotics. An interesting phenomenon concerning the toxicity of Cd is the role that metallothioneins play. Metallothioneins are nonenzymatic proteins and are ubiquitous in the animal kingdom. The proteins are rich in cystein and are capable of binding metals such as Cd, thus alleviating its toxicity.

Inorganic gases, including SO_2, NO_2, O_3, and CO, as they relate to air pollution are covered in Chapter 8. The sources, characteristics, and effects on plants, animals, human health, and biological effects are presented in this chapter. Among the nitrogen oxides, the importance of nitrous oxide (N_2O) is also addressed. N_2O has recently been shown to be an extremely important gas in contributing to stratospheric ozone layer depletion. In Chapter 9, "Air Pollution: Particulate Matter," discussion begins with physical and chemical formations of this type of air pollutant, followed by its influence on health. Special emphasis is placed on the occurrence and health effects of silica and silicosis, asbestos and asbestosis, and beryllium and berylliosis.

The sources and forms of fluorine found in the environment and how they have an impact on the health of living organisms are discussed in Chapter 10, "Environmental Fluoride." Even though fluoride is not listed by the Environmental Protection Agency (EPA) as one of the six "criteria air pollutants," it is nevertheless an important air pollutant. It is known that fluorine has an impact on tens of millions of people throughout the world, particularly in the less-developed countries. As an air pollutant, fluoride is known to be most phytotoxic and can damage plants at a very low concentration. In addition, it is an important waterborne pollutant because at an elevated level it is hazardous to both humans and animals. The chapter begins with how fluoride is produced as a result of different industrial processes. This is followed by discussion of the chronic and acute effects of fluoride on plants, animals, and humans and the biochemical effects on these organisms. The relationship between nutrition and fluoride toxicity is also discussed.

The volatile organic compounds (VOCs) presented in Chapter 11 are another important group of air pollutants. A large number of VOCs are emitted from industrial and nonindustrial facilities in the United States and the world. Chemically, VOCs include both aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, some alcohols, esters, and aldehydes. Both natural and anthropogenic sources contribute to VOC emissions. Natural sources include petroleum, forest fires, and the transformation of biogenic precursors, whereas anthropogenic sources include high-temperature combustion of fuels, emission from crude and refined oil, municipal incineration, emissions from power boats, and burning of crops before or after harvesting as an agricultural practice. The sources of exposure and health effects, including carcinogenic impacts, of polycyclic aromatic hydrocarbons (PAHs) and their metabolism are presented. In addition, the controversies related to the use of bisphenol A (BPA) are discussed.

Chapter 12 covers the environmental metals and metalloids involved in soil and water pollution. The chapter contains the characteristics and uses of lead (Pb), cadmium (Cd), mercury (Hg), nickel (Ni), and arsenic (As). Because of its importance as an air pollutant, Pb has been included as one of the six criteria air pollutants designated by the EPA. Lead poisoning is the most common and serious environmental disease affecting young children in the United States. The characteristic health and biological effects of the metal are discussed in some detail. The biochemical effect of Pb, particularly its inhibitory effect on heme biosynthesis, is discussed, and the relationship of nutrition with Pb toxicity is also covered. Two well-known outbreaks termed *itai-itai-byo* and “Minamata disease” were caused by Cd and methyl Hg poisonings, respectively. Their toxic effects on plants, animals, and humans are reviewed in this chapter, together with the alleviating effect of nutrition. The chapter also discusses the occurrence and health effects of Ni and As, including their carcinogenic effects.

Chapter 13 is about pesticides and related materials. It discusses the chemistry, characteristics, and health effects of several representative groups of pesticides and herbicides. In addition, several halogenated hydrocarbons, such as polychlorinated biphenyls (PCBs) and dioxins, are reviewed. These have become of much concern in recent years. The review covers chlorinated hydrocarbons and organophosphorus compounds. In the discussion of herbicides, 2,4-D (2,4-dichlorophenoxy acetic acid) and 2,4,5-T (2,4,5-trichlorophenoxy acetic acid), PCBs, polybrominated biphenyl, and dioxin (2,3,7,8-tetrachlorobenzo-*p*-dioxin) (TCDD) are stressed, including their effect on gene regulation.

Chapter 14 deals with occupational toxicology. The discussion traces a short history of different occupational diseases beginning with the pre- and postindustrial revolution, followed by toxicology and preventive medicine in the modern era. Special emphasis is placed on respiratory toxicity, irritation of airways and edema, occupational respiratory diseases, diseases caused by sensitizers, and nanoparticles, as recent chemicals of concern.

One of the most pressing environmental issues facing environmental toxicology is endocrine disruption. This general concept is discussed in Chapter 15. The general perception on this issue is that exposure to certain anthropogenic chemicals that can interact with and disrupt the endocrine system may cause some form of malfunction and ultimately pose serious health problems in humans and wildlife, fisheries, or their progenies. Chemicals that can induce endocrine disruption are called endocrine disrupters (EDs) or endocrine-disrupting chemicals (EDCs). The chapter begins with a brief review of hormonal function. This is followed by a discussion of the characteristics of EDs and their mode of action. Several examples of endocrine disruption are then reviewed. They include induction of developmental toxicity, estrogen mimics, induction of sterility, imposexes, antiandrogens, hypothyroidism, and hormonal cancers. A number of methods have been developed and used for studying the presence and action of EDs. A widely used method of vitellogenin measurement together with enzyme-linked immunosorbent assay (ELISA) technique is discussed.

Chapter 16 presents mutagenic pollutants. Mutation is a process in which the hereditary constitution of a cell is altered, ultimately leading to a genetically altered population of cells or organisms. Mutagen is the agent that causes mutation. Although mutations can occur in the RNA of viruses and the DNA of cytoplasmic organelles, the mutations of greatest interest occur within genes in the nucleus of the cell. This chapter reviews mutagens that are commonly found and that are of most concern to humans. Included in the discussion are ultraviolet light, ionizing radiation, microtoxins, and organic and inorganic chemicals. The way in which these agents interact with DNA to cause mutation, leading to carcinogenicity, are reviewed.

The last chapter of this book, Chapter 17, presents information on environmental cancer. Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. In recent years, there has been a growing concern about the possible effects of a large number of environmental toxicants on carcinogenesis, the production of cancer. Studies showed that nearly 30% of the total mortality in many industrialized countries is attributed to cancer. The chapter covers cancer causes, stages in development, metastasis, and mechanism of chemical carcinogens. In chemical carcinogens, such chemicals as free radicals, DDT, vinyl chloride, alkylating agents, trichloroethylene (TCE), and PAHs are discussed. A special emphasis is placed on the increase in respiratory cancer death rates in the United States. DNA repair is the topic at the end of the chapter.

2 Environmental Changes and Health

2.1 OUR CHANGING ENVIRONMENT

2.1.1 Introduction

Our environment has been changing dramatically in recent decades. Important factors contributing to the changes include a growing world population, global warming, expanding industrialization, and advancing technology and economics. Some other changes are worsening air and water pollution, mounting solid waste, acid rain, depletion of the ozone layer, and increasing endocrine disrupters in the environment. These changes have profound impacts on the health and well-being of living organisms. Some of the major issues are discussed here.

2.1.2 World Population

The world population has been increasing steadily and reached about 6.8 billion in 2010. The increases differ from country to country, however. The United States Census Bureau projected that China and India alone account for 37% of the world's population, and that with China's overall population growth rate being slowed to 0.5% annually, the projected peak is 1.4 billion people, lower than previously estimated. In comparison, India's annual growth rate is 1.4%, which is attributed to the fertility rate of 2.6 births per woman, compared to China's fewer than 1.6 births per woman. Furthermore, with almost 1.2 billion people, India is disproportionately young; roughly half the population is younger than 25. It is estimated that in the coming decades, India is projected to surpass China as the world's most populous nation, with estimates ranging from 1.5 billion to 1.9 billion people (Roberts 2009).

India's leaders recognize that this must be avoided. Many researchers also consider that it is high time for India to act. To cope with the serious situation, different programs to slow birth rates are being tried in India. For example, a pilot program has been initiated in Satara, a city in the state of Maharashtra. The city has provided cash bonuses to young women to slow birth rates (Yardley 2010).

In contrast to both China and India, Russia has been experiencing a declining population in recent years. According to projections by the United Nations, Russia's population, currently 140 million, will likely be decreased to 116 million by 2050 (*The Wall Street Journal*, September 11, 2010), a decrease of more than 17%, or more than 4% a year.

2.1.3 GLOBAL CLIMATE CHANGES: GLOBAL WARMING

According to the U.S. National Oceanic and Atmospheric Administration (NOAA), the global tropospheric temperature for 1978 to 2002 increased 0.22 to 0.26°C per 10 years. The increase was consistent with the global warming trend derived from surface meteorological stations (Vinnikov and Krody 2003).

A recent *New York Times* report showed that the icecap atop Mount Kilimanjaro in Tanzania is retreating at such a pace that it will disappear in less than 15 years. In addition, its glaciers are also rapidly thinning, with one spot having lost nearly 1 m of thickness since early 2002. Some scientists report that the mountain has lost 82% of the icecap it had in 1912, when it was first carefully surveyed. The summits of both the Northern and Southern Ice Fields atop the mountain have thinned by 1.9 m and 5.1 m, respectively. At 5,896 m high, the mountain is one of Tanzania's top tourism draws. It brings in an estimated US$50 million a year, a revenue that is now under threat.

Climate changes have also been shown to affect ocean temperature, salinity, and flow patterns. Warmer temperatures weaken the ice, making it vulnerable to current changes and other forces. Scientists think that this has already influenced the stability of ice shelves in the Antarctic. Indeed, two chunks of ice, the size of a small country, broke off the Antarctic Peninsula's Larsen Ice Shelf in 1995 and 2002, respectively (Kaiser 2003).

Only 100 years ago, the whole northern coast of Ellesmere Island was edged by a continuous ice shelf. About 90% of the shelf is now gone. Existing records show an increase of 0.4°C every 10 years since 1967, and the average July temperature has been 1.3°C since that year (Burton 2001). Under the most extreme scenario, global warming could thaw the top 3.3 m of permafrost near the ground surface in most areas of the Northern Hemisphere by 2100, altering ecosystems across Alaska, Canada, and Russia, on a scale unseen for thousands of years. An ice chunk four times the size of Manhattan broke off a Greenland glacier. The Petermann glacier cracked early in August 2010, creating the biggest arctic ice island in half a century (*USA Today* 2010). The chunk of ice 100 miles square is a reservoir of freshwater that, if collapsed, would raise global sea levels by a devastating 6 m. Researchers are scrambling to plot the trajectory of the floating ice shelf, which is moving toward the Nares Strait separating Greenland's northwestern coast and Canada's Ellsemere Island.

The *Anchorage Daily News* (O'Hara 2005) also reported that warming temperatures could melt the top 11 feet (3.3 m) of permafrost in Alaska by the end of the century. This will damage roads and buildings with sinkholes, transforming forest and tundra into swamps, and releasing large amounts of greenhouse gases into the air. This meltdown forecast comes amid other signals that the Arctic climate has been changing fast: shrinking sea ice cover, warmer temperatures, and shifting vegetation. According to *The New York Times* (Gillis 2010), scientists are trying hard to answer one of the most urgent, and most widely debated, questions facing humanity: How fast is the world's ice going to melt? Many scientists now consider that sea level is likely to rise perhaps 0.9 m (3 feet). Others suggest that the rise could conceivably be double that figure. A rise of even 0.9 m would inundate low-lying lands in many countries, rendering some areas uninhabitable. For example, in the United States, parts of the East Coast and Gulf Coast would be hit hard. And, some of the world's great cities, such as London, Venice, Cairo, Bangkok, and Shanghai would be critically endangered.

Another recent study also showed that the Pine Island Glacier in Antarctica is accelerating its retreat. Glaciologists are not sure what caused the retreat. Together with policy makers, they wonder whether it is because of global warming, the ozone hole, or simply a random variability.

Onboard a cruise ship, one of the authors of this volume (Yu) visited the Antarctic Peninsula in February 2010. He saw countless ice chunks and ice-sheets floating in the seawater. Figure 2.1 shows a picture taken from the ship.

Many environmental researchers believe that the burning of fossil fuels is slowly causing the climate to change. Exhaust from the fuel burning increases the level of CO_2 and NO_x and particulate matter in the atmosphere. This in turn causes Earth to retain heat, warming the globe. The CO_2 level in the atmosphere is already dangerously high. The current level is reported to be 384 ppm and rapidly rising. According to the Intergovernmental Panel on Climate Change (IPCC), an atmospheric CO_2 level of 540 to 970 ppm and a global temperature rise of 1.4 to 5.8°C could occur by 2100. Similarly, scientists around the world have found that climate change is altering natural ecosystems, making profound changes in the ways that animals live, migrate, eat, and grow. While some species have benefited from the shift, others have been left disastrously short of their food supply. Some are known to have simply disappeared. Many scientists consider that, if warming continues as predicted, 20% percent or more of the planet's plant and animal species could be at increased risk of extinction (Hogue 2010).

Meanwhile, Russia battled drought and wildfires while sweltering in record heat that claimed thousands of lives. In Pakistan, flooding caused displacement of millions of Pakistanis. The eastern United States chalked up record numbers of days with high temperatures. According to the World Meteorological Organization (WMO),

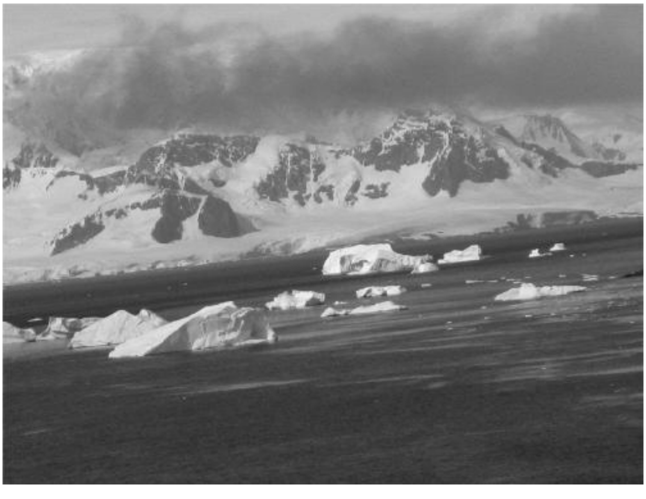

FIGURE 2.1 Ice chunks and ice sheets floating in Antarctica seawater. (Picture by M. H. Yu, February 2010.)

all of these events are extreme—they compare with or exceed previous records for intensity, duration, or geographic extent.

The melting glaciers in the state of Washington are also causing concerns. With more glaciers than any state in the lower 48, Washington has emerged as a leader in demonstrating global warming. A national environmental group recently reported that the North Cascades and Mt. Rainier are among the dozen national parks most susceptible to climate change. According to the National Park Service at Mt. Rainier, which is among the best-studied sites in the nation, the area covered by glaciers shrank by more than a fifth from 1913 to 1994, and the volume of the glaciers by almost one-fourth from 1912 to 2001; the Nisqually Glacier on Mt. Rainier retreated nearly 1.6 km.

According to a recent U.N. Convention of Biodiversity report (UN News Service 2010), the globe falls short of biodiversity. The report found that far too many of the world's plants and animals—and the wild places that support them—are at risk of collapse, despite a global goal set in 2002 for major improvement by 2010. The report showed that the species most at risk of extinction are frogs and other amphibians, and that coral reefs are the species deteriorating most rapidly; the survival of nearly a quarter of all plant species is threatened. Pollution, climate change, drought, deforestation, and overfishing are among the culprits named by the agency in May 2010.

2.1.3.1 Impact on Plants

The U.N. Convention of Biodiversity report was echoed by the observation of Weis at the University of Toronto (Franks et al. 2007). He showed that the field mustard weed has responded to repeated recent droughts in California—believed to be connected to climate change—by flowering earlier in the year and producing strong seeds before the soil dries out in the summer. The reproductive cycle of the weed has also sped up, allowing the plant to respond faster to the changing climate. In general, species that can reproduce rapidly will adapt more easily to the pace of climate change than old trees. According to Randolph E. Schmid of the Associated Press, global warming apparently drives plants to higher ground (Schmid 2008). A study of 171 forest species in Western Europe showed that most of them are shifting their favored locations to higher, cooler spots. For the first time, research can show the "fingerprints of climate change" in the distribution of plants by altitude, not only in sensitive ecosystems. The researchers pointed out that the quickest to relocate were plants such as herbs, ferns, and mosses with shorter life spans and faster reproduction cycles. In contrast, long-lived plants like trees that reproduce slowly are more threatened by climate change because they cannot quickly relocate.

2.1.3.2 Impact on Birds and Animals

A new assessment showed that a quarter of the world's wild mammal species are at risk of extinction. The new assessment, which took 1,700 experts in 130 countries 5 years to complete, covered all 5,487 wild species identified since 1500 and indicated that "mammals are definitely declining, and the driving factors are habitat destruction and over harvesting," according to Jan Schipper, a lead author of the global mammal assessment of the International Union for the Conservation of Nature (IUCN 2009). The researchers concluded that 25% to 36% of the mammal species are threatened with extinction. Land and marine mammals face different threats, and large mammals are more vulnerable than small ones. For land species, habitat loss and hunting represent the greatest danger; marine mammals are more threatened by accidental killing, ship strikes, and pollution. Primates face some of the most intense pressures: According to the survey, 79% of primates in South and Southeast Asia are facing extinction.

Researchers reported that autumn temperatures in the Arctic are at record levels, 9°(F) above normal. The Arctic Ocean is getting warmer and less salty as sea ice melts, and reindeer herds appear to be declining.

A unique observation has been made. In the summer of 2009, researchers from the Imperial College London noticed that the wild Soay sheep off the western coast of Scotland had shrunk. On average, they have become 5% smaller (Walsh 2009). This was surprising because bigger is generally better for sheep. They fatten up on grass during the fertile, sunny summer; when the harsh Scottish winter comes, the grass disappears, and the smallest, scrawniest sheep tend to die off while their heftier, fitter cousins survive to reproduce in the spring. Researchers considered that it is not that evolution has been repealed in Scotland. They think rather that global warming has simply made it easier for smaller, less-fit Soay sheep to survive. And, plenty of other species are quickly adapting to the changing climate in smaller ways. Bryan Walsh reported that as the planet warms, species like the wild Soay sheep are evolving in response. They have been getting shorter and milder, largely as a result of climate change. That makes food more abundant and allows some of the smaller, younger sheep not only to survive but also to have offspring that tend to be tiny, yet have a better chance of survival because of the warmer winters. But, they may not keep pace with the astonishing speed of climate change (*Time*, 2009).

Tim Coulson and his colleagues at the Imperial College London found the surprising fact about evolution and global warming, but they also recognized that the relationship is not linear. It is not only rising temperatures that trigger evolution but also changing seasonal patterns, especially among species that live in the temperate or polar regions and are finely tuned to the seasons. Earlier springs and later falls confuse wildlife, which tell the time of year by the length of the days. They are using the most reliable environmental cue they have: "light," said Bradshaw and Holzapfel (2001), at the University of Oregon. Holzapfel considered that means big shifts in fundamental survival behavior. As the environment changes, individuals that cannot change are lopped off. "What's left is a different kind of population that can evolve and move forward." Global warming may outrun even the fittest wildlife, and the short-term success of animals like the Soay sheep may not last. The outcome of when evolution cannot keep pace with climate change is "extinction," unfortunately.

A report by the Interior Department showed that changes in the global climate are imposing additional stress on hundreds of species of migratory birds in the United States that are already threatened by other environmental stressors. The latest version of the department's annual "state of the birds report" showed that nearly a third of the nation's 800 bird species are endangered, threatened, or suffering from population decline. For the first time, the report added climate changes to other factors threatening bird populations, including destruction of habitat, hunting, pesticides, invasive species, and loss of wetlands (Broder 2010). The report indicated that oceanic and shorebirds are among the most vulnerable to climate change because of rapidly changing marine ecosystems and rising sea levels. Goose populations are increasing as they expand their range within the Arctic. NOAA reported that the surface of the ocean is growing warmer, and record temperatures were set (NOAA 2010). Kenneth Rosenberg, director of conservation science at Cornell University's Lab of Ornithology, reported that "birds are excellent indicators of the health of our environment, and right now they are telling us an important story about climate change" (Rosenberg 2010).

2.1.3.3 Impact on Tropical Species

While the most significant harm from climate change so far has been in the polar regions, tropical plants and animals may face an even greater threat. Researchers at the University of Connecticut (Colwell 2008) warned that some tropical species are living near their maximum temperatures already, and warmer conditions could cause them to decline. The researchers estimated that a temperature increase of 3.2°C (5.8°F) over a century would make 53% of the 1,902 lowland tropical species they studied subject to attrition. In addition, the tiny Kihansi spray toad, which once numbered at least 17,000 at Kihansi Falls in Tanzania, has been shown to be extinct in the wild.

2.1.3.4 Impact on Freshwater Fish

According to the IUCN, which lists over 47,000 of the world's species, more than 1,000 freshwater fish species are threatened with extinction, reflecting the strain on global water resources. Overall, the 2009 survey found that over a third, or 17,292 species out of 47,677 assessed, are now in danger of extinction (*The Straits Times (Singapore)*, 2009).

2.1.4 IMPACT ON MARINE OXYGEN

According to the Associated Press, some researchers warn that low-oxygen zones where sea life is threatened or cannot survive are growing as the oceans are heated by global warming. Oxygen-depleted zones in the central and eastern equatorial Atlantic and equatorial Pacific oceans appear to have expanded over the last 50 years.

Low-oxygen zones in the Gulf of Mexico and other areas have also been studied in recent years, raising concerns about the threat to sea life. Continued expansion of these zones could have dramatic consequences for both sea life and coastal economies. Most marine species have a minimum oxygen threshold that they need for survival. As oxygen levels decrease, these animals will suffer or be compelled to move to other areas. Over time, the optimal area for various species will be compressed. The general pattern is for colder ocean waters in the north and south to absorb oxygen, cool and sink below the surface, and then flow toward the equator.

Scientists reported that 2007 was the warmest year on record in the Arctic (Rice 2008), resulting in a record loss of sea ice. The sea ice melt in 2008 was second only to 2007. Rising temperatures help melt the ice, which in turn allows more solar heating of the ocean. That warming of the air and ocean affects land and marine life and reduces the amount of winter sea ice that lasts into the following summer. The study also noted a warming trend in Arctic and increase in greenness as shrubs move north into areas that were formerly permafrost. The Arctic Ocean continued to warm and freshen due to ice melt. This was accompanied by an “unprecedented” rate of sea-level rise of nearly 0.1 inch per year. Warming continued around Greenland in 2007, resulting in a record amount of ice melt. The Greenland ice sheet lost 24 cubic miles of ice, making it the largest single contributor to global sea-level rise.

Some scientists are concerned about an even more worrisome effect on future generations. With the long residence time of CO_2 in the atmosphere and warmer oceans, what are the prospects for the twenty-second century? Many scientists consider that, because of their wealth and advanced technology, the United States and other industrial nations may be able to cope with global warming effects in their own lands in this century but are unlikely to escape serious impacts in the following century (Burton 2001).

Knowledge about the contribution of CO_2 and other greenhouse gases to global warming has led a number of countries to lower their emissions. This trend is particularly marked in several European countries, such as Germany, France, Italy, and the United Kingdom. By contrast, some Asian countries, including China, India, and South Korea, have markedly increased their energy-related carbon emissions over the past three decades. An annual report by the International Energy Agency showed that global energy use will grow 36% by the year 2035, spurred mostly by China’s rapid increase in energy consumption. According to the report, China overtook the United States in 2009 to become the largest energy user in the world, and its per capita consumption—currently one-fifth that of the United States—is expected to rise over the coming decades, with automobile use projected to increase 10-fold (*Time*, November 22, 2010, p. 21).

2.1.5 RISING ACIDITY OF SEAWATER

According to a panel of marine scientists in Seattle, Puget Sound faces an uncertain future due to the increasing acidity of seawater. These changes are coming more rapidly than expected and could disrupt food chains and threaten the shellfish industry in Washington (BellinghamHerald.com, May 28, 2008). The acidic seawater is moving closer to shallow waters containing the bulk of marine life. The increasingly corrosive waters threaten the survival of many marine organisms. The latest research showed that acidic water is appearing along the Pacific Coast decades earlier than expected. The acidified water does not pose a threat to humans, but it could dissolve the shells of clams, oysters, and other shellfish. The state of Washington is known to produce 85% of all shellfish on the West Coast.

According to the Associated Press, Victoria, British Colombia’s capital, plans to stop pouring a huge volume of untreated sewage into the marine waters between Vancouver Island and Washington State. Regional politicians approved a $1.2 billion plan to build four treatment plants by 2016 to handle about 34 million gallons (about 130 million liters) of raw sewage that Victoria and six suburbs pump into the Strait of Juan de Fuca each day. The cities are home to about 300,000 people.

Global ocean currents make the Pacific Northwest’s coastal ecosystems particularly vulnerable to acidification effects. A worldwide “conveyor belt” slowly carries colder water from the North Atlantic to the North Pacific. Along the way, the water accumulates CO_2 from the dead organisms, so it naturally has a higher CO_2 concentration before human-made CO_2 is added. A process known as "upwelling" drags this water into shallower, coastal areas. As long as CO_2 continues to increase in the atmosphere, the oceans will continue to absorb that, but what we are seeing is only going to get worse. Some scientists indicated that even though we will not see a total collapse in food chains, we will see substitutions; we may end up with food chains or food webs that are highly undesirable and not productive for the means that we use them today.

Increased use of fossil fuels has caused the levels of CO_2 in the atmosphere to nearly double since the Industrial Revolution. According to Richard A. Feely (Feely et al. 2004), a senior scientist with the NOAA's Pacific Marine Environmental Laboratory in Seattle, "Over the past 200 years the oceans have absorbed approximately 550 billion tons of CO_2 from the atmosphere, or about a third of the total amount of anthropogenic emission over that period." That means the ocean currently absorbs about 22 million tons of CO_2 per day, he added. The high levels of human-generated CO_2 are the main reason that oceans are acidifying. The global pH of surface ocean waters is currently about 8.1 and is expected to drop by approximately 0.3 units in the next 50–100 years. As the ocean becomes more acidic, scientists anticipate myriad changes to the ocean's chemistry. Changing pH is likely to affect many aspects of biochemistry, development, and reproduction for many marine organisms. The scientists recognize that ocean acidification threatens marine ecosystems, but few studies have examined effects on fish. This acidity dissolves $CaCO_3$, which constitutes shells. If diatoms, corals, clams, and oysters succumb to this, it will wipe out not only the shellfish industry but also potentially the entire marine food chain.

Scientists have been concerned for many years that lower ocean pH caused by absorption of emitted CO_2 could decrease calcification processes underlying the growth of shells and the hard exteriors of corals. Scientists are also looking into some unexpected consequences of ocean acidification, such as disruptions to sound propagation and transmission of chemical cues. For example, Philip L. Munday of James Cook University in Australia and colleagues raised clownfish (*Amphiprion percula*) in seawater acidified with CO_2 (Munday et al. 2011). At pH 7.8, a condition that could arise around 2100 if the oceans continue to absorb CO_2 at the current rate, the fish lost the ability to distinguish between chemical cues that might help them locate a proper habitat. At pH 7.6, the fish did not respond to any environmental cues. If the pH drop is widespread, it could threaten the survival of a broad range of marine species. More studies are needed to see whether the effect is reversible. Another example was given in a recent *C&EN* report indicating that ocean acidity affects fish senses (*C&EN,* February 9, 2009). Some scientists believe that the net effect of these and other yet-undiscovered changes may threaten the survival of a wide variety of marine organisms.

The Environmental Protection Agency (EPA) stressed that states should consider acting against ocean acidity. It advised that states with coastal water that is becoming more acidic because of CO_2 should be listed as impaired under the Clean Water Act (TheBellinghamHerald.com, May 24, 2010). The federal agency's memo to states recognized CO_2 as not only an air pollutant but also a water pollutant and noted the serious impacts that ocean acidification can have on aquatic life. Currently, about 40,000 bodies of water are listed nationwide as impaired.

2.1.6 Rise in Diseases

Another concern about the impact of global warming is the possible rise in diseases. For instance, a variety of diseases broke out in several countries during the 1990s after extraordinary heat followed by drastic weather conditions, such as heavy monsoons and floods. As a result, significant numbers of deaths occurred worldwide induced by diseases such as cholera, pulmonary hantavirus, plague, and dengue fever. Some scientists cautioned that perhaps an even more immediate threat of the warming trend is the rapid spread of disease-bearing bugs and pests (Linden 1996).

A U.N. report predicted that global temperature would rise by between 2°C and 4°C by the end of this century. The best estimate is that if emission of the greenhouse gases continues to rise, the global average temperature may rise 3°C by 2100 (UNEP 2011).

2.2 AIR POLLUTION

2.2.1 Introduction

Air pollution is generally defined as the presence of substances in air at such concentrations, duration, and frequencies that it causes adverse effects on the health of living organisms and the environment. The problems related to air pollution have increased steadily since the end of World War II. The extent to which air pollution influences public health is shown by many air pollution-related episodes. One of those episodes is the widely known 4,000 "excess deaths" that occurred in London in 1952 (see Figure 3.1). Similar but less-serious air pollution-related injuries also occurred in other major cities in the world, including Osaka, Los Angeles, and New York, although the air pollutants involved were often different from one another.

A wide range of pollutants is present in indoor and outdoor air. They include SO_2, NO_x, CO, and O_3 and other photochemical oxidants, different types of particulates, heavy metals such as lead, and various kinds of volatile organic compounds (VOCs). The major sources of air pollution are combustion of fossil fuels for electricity and transportation, a variety of industrial processes, heating, and cooking.

According to the North American Commission for Environmental Cooperation (CEC), in the United States, electric power plants accounted for one-quarter of the industrial pollution released into the North American environment in 1998. This was closely followed by pollution from the primary metals sector, the chemical industry, and hazardous waste management sectors (Benner 2001). While problems associated with air pollution remain of global concern, encouraging results have been shown with its control in the United States and other industrialized countries. For example, according to an EPA report, a substantial improvement in air pollution has occurred in the United States since 1970. Emissions of six principal air pollutants (i.e., SO_2, NO_x, CO, O_3, particulate matter, and lead) have declined 48% since 1970. SO_2 emissions from power plants are 9% lower than in 2000 and 41% lower than in 1980, while NO_x emissions declined 13% from 2000 and 33% from the 1990 level. The levels of ground-level O_3, however, have decreased the least. The 10-year trend has been relatively unchanged. According to a report by the EPA, total annual emission of 188 regulated toxics in the United States has declined by 36% since 1980 (USEPA 2010). Still, there are some unacceptable risks posed by industrial air pollution in some parts of the country.

2.2.2 Air Pollution and Developing Economies

Many of the rapidly growing cities in the world are experiencing growing air pollution problems. Serious concerns have been voiced about the health hazards of air pollution in a number of less-developed countries. With unprecedented growth shown in urban centers, megacities with populations of 10 million or more have emerged in many less-industrialized countries, including China and India. In India alone, for example, there are four such cities, with three others expected to join the ranks in the next 20 years. The majority of the 300 million urban dwellers in India, representing 30% of the country's population, are experiencing deteriorating air quality. India's major cities are reportedly among the most polluted in the world, with concentrations of several air pollutants well above the levels recommended by the World Health Organization (WHO). Some scientists in the country caution that the residents of its megacities face significant risks to their health from exposure to air pollutants (Kandlikar and Ramachandran 2000).

China's Environmental Protection Administration acknowledged early in June 2010 that, despite tougher measures, pollution is increasing as the country's economy rebounds, quashing hopes that China had turned a corner in 2009 when emissions dropped. Emissions of SO_2, for example, rose 1.2% in the first quarter compared with 2009, as exports and domestic demand picked up and China burned more coal in power plants and factories, according to Zhang Lijun, vice minister of the environment in the country. He also indicated that authorities now are "not very optimistic" on the prospects of emission cuts. Ground-level ozone (O_3) is formed by reactions between CO and other pollutants and sunlight. O_3 irritates the respiratory system and may increase the risk of heart diseases. The country's EPA authorities estimated the annual number of premature deaths in China caused by air pollution at 358,000 (TheBellinghamHerald.com, June 4, 2010).

As is widely known, China has achieved extreme economic growth for the past several decades. The growth is coupled with accelerated industrialization, greatly increased energy consumption, and urbanization (He et al. 2002). The accelerated urbanization is evidenced by marked increases in the proportion of urban population to the total population in China, from 18% in 1978 to 31% in 1999, a growth rate three times the world average during this period. The explosive economic growth also made China the world's largest energy consumer. Coal accounts for roughly 25% of the world energy supply and 40% of the carbon emissions (http://energy.gov/carbongraph). Coal is the primary energy source in China, accounting for about 80% of the total energy consumption. In China, the use of coal is the origin of many air pollution problems, such as SO_2 pollution, particulate matter, and acid rain (He et al. 2002).

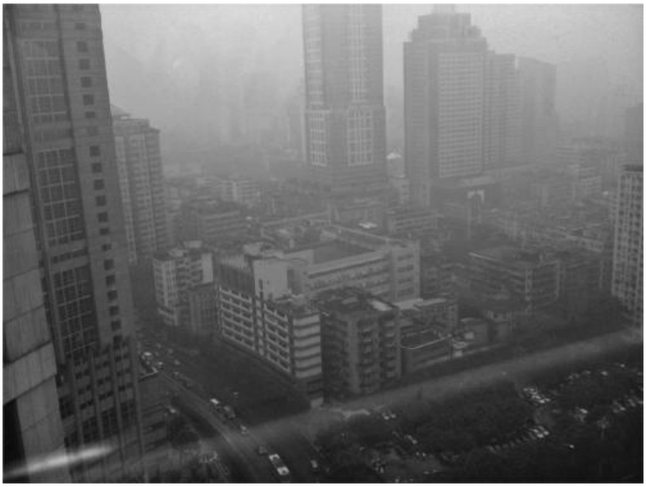

FIGURE 2.2 A smoggy day in Guangzhou, China. (Courtesy of C. Y. Yu, October 2010.)

Furthermore, crude oil consumption has also increased, with an average growth rate of 6% per year in the past decades. Part of this increase is the result of growing use of motor vehicles. This has accelerated ambient pollution by NO_x, CO, and other pollutants in large cities. Indeed, China's growing energy consumption, reliance on coal, and rapidly increasing use of vehicles place a heavy burden on the urban atmosphere, and urban air pollution has become a major environmental problem in the country. Many cities have suffered from increasingly serious air pollution since the 1980s. During the 1990s, some megacities, such as Beijing, Shanghai, Shenyang, and Guangzhou (Figure 2.2), were always listed among the top 10 most polluted cities in the world.

According to Tom Friedman, a columnist with *The New York Times*, one of his friends living in Beijing wakes up every morning and does his own air quality test—as many Beijing residents do. He looks out his 24th-story window and checks how far he can see. On a rare pristine day, when the wind has swept Beijing, he can see the Fragrant Mountain rising to the northwest. On a "good pollution day," he can see the China World building four blocks away. On a bad day, he cannot see the building next door (TheBellinghamHerald.com*,* November 20, 2006).

Some researchers in China have expressed serious concerns about the public health effects of urban air pollution in that country (He et al. 2002). The concerns were strongly supported by the studies of Xu et al. (1995). They concluded that the existing air pollution levels in Beijing are associated with adverse health outcomes. The scientists studied the data on the average number of daily hospital outpatient visits at a community-based hospital in Beijing and compared the data with the levels of SO_2 and total suspended particles (TSP) in the atmosphere. They found that increases in the levels of the two types of pollutants were significantly correlated with the increase in visits to local internal medical clinics in both winter and summer.

The effects of air pollution affecting human health were also reported by scientists in Seoul, South Korea. For example, Ha et al. (2003) studied the effect of air pollution on mortality among postneonates, those aged 2 to 64 years, and those over 65 years of age. The study included daily counts of total deaths and deaths due to respiratory problems, along with analyses of daily levels of particulate matter less than 10 μm (PM_{10}). The results showed that children's mortality rates, particularly those related to the respiratory system, were most correlated with PM_{10}.

According to Pan Yue, China's vice minister for environmental protection, "It will only come from a shift of attitudes from the very top to the very bottom (Economy 2007). My job is to educate and encourage this shift, so that officials don't just think about economic growth as GDP [gross domestic product] growth but also factor in environmental health." China's tenth 5-year plan, which began in 2000, called for a 10% reduction in the SO_2. When that plan concluded in 2005, air pollution in China is said to have increased by 27%.

In its first report on China's environment, the International Organization for Economic Cooperation and Development (OECD) found that the country is harming its environment and the health of its citizens as its economy leaps forward (Tremblay 2007). OECD estimated that unless the current trend is changed, by 2020 air pollution will lead to 600,000 premature deaths annually in Chinese cities. Citing data from the Chinese Ministry of Health, OECD said 300 million rural residents already lack access to safe drinking water. The report also indicated China consumes resources inefficiently. It found that the country generates more pollution and consumes more resources per unit of GDP than OECD average. "China is aiming to quadruple its GDP between 2000 and 2020, but the country requires commensurate strengthening of environmental management and finance so that economic growth is environmentally sustainable," the report said.

In the richer parts of China, local officials are responding to citizens' demands for less pollution. But in much of the country, the report said, one of the primary obstacles to progress is that local leaders have to raise revenues locally and face "limited accountability." As a result, economic growth takes precedence over environmental preservation. OECD recommends improving the enforcement capabilities of China's local Environmental Protection Bureaus and turning the state Environmental Protection Agency into a ministry. The report noted that the state of China's environment is an international issue because it involves global energy consumption patterns, global manufacturing patterns, and transboundary pollution. The conclusions and recommendation in the report were approved by a Chinese government delegation that took part in the project.

In 2007, the WHO called on governments to improve air quality in their cities because air pollution prematurely kills 2 million people a year, with more than half the deaths in developing countries (TheBellinghamHerald.com, August 26, 2007). Reducing pollution from particles that are too small to be filtered in the nose and throat and settle in the lungs could save as many as 300,000 lives every year, according to the regional office of WHO in Manila. And, reducing levels of those extremely small particles could cut the deaths from air pollution by about 15%. More than 10 million people are at risk for lung infection, cancer, and shortened life expectancy because they live in the 10 worst-polluted cities in the world, according to the report.

The National Health Bureau in Taiwan reported a similar observation. The report showed that men exceeded women in 9 of the 10 most serious disease deaths, and life span for men was 6.6 years less than that of women. The death rate was 748.6 per 100,000 for men compared to 481.9 per 100,000 for women. Of these, the cancer death rate for men was 33.6% higher than that for women. High blood pressure was the top preventable contributing factor to the deaths, followed by cigarette smoking, physical inactivity, and being underweight. Lung cancer was the top cause of death in that disease category, and 63% of the men surveyed were smokers.

The 2006 Environmental Performance Index, jointly produced by Yale and Columbia universities, shockingly ranked the United States 28th in overall performance, behind most of Western Europe, Japan, Taiwan, Malaysia, Costa Rica, and Chile but ahead of Russia and South Korea (TheBellinghamHerald.com, January 23, 2006).

2.3 INDOOR AIR POLLUTION

Indoor air pollution is one of the top five most urgent environmental risks to public health, according to the U.S. EPA. Risk can rise in winter, as people tightly shut the windows and doors and unwittingly trap unhealthy air inside (BellinghamHerald.com, November 20, 2006). Poor indoor air quality can exacerbate chronic lung diseases such as asthma. It also can cause headaches, dry eyes, nasal congestion, nausea, and fatigue.

Major sources of indoor air pollution include tobacco smoke, mold that grows in poorly ventilated basements and bathrooms, smoke from wood-burning fireplaces and stoves, fumes from housecleaning products, smoke and gas fumes from cooking, dust trapped in carpets, cancer-causing radioactive radon gas, and CO that can result from poorly vented heaters and other fuel-burning appliances. CO can affect human health even at low levels. Nearly 300 Americans die each year from CO exposure, but less than 30% of those people have detectors at home.

2.4 WATER POLLUTION

Historically, the concern about water pollution was related to its health effects. While in many countries this remains true, in the United States and other developed countries the results of improved treatment and distribution methods have, to a certain degree, shifted the emphasis. Many citizens in these countries generally regard water pollution not so much in terms of health, but rather of conservation, aesthetics, and the preservation of natural beauty and resources. Many of the world's lakes, rivers, and streams have suffered from the effects of water pollution. Moreover, the problems associated with water pollution are worsening in many countries, particularly in some of the less-developed countries.

The main sources of water pollution include both inorganic and organic wastes, heat from industries, petroleum compounds, municipal wastes, agricultural wastes, pesticides, and acid mine drainage. Many industrial processes have the potential of discharging different types of wastes that could cause significant water pollution problems.

Human diseases and casualties arising from water pollution attracted worldwide attention after "Minamata disease" and *itai-itai-byo* ("ouch-ouch disease") broke out in Japan during the 1940s and 1950s. Minamata disease was caused by eating fish and shellfish laden with highly toxic methylmercury, while itai-itai-byo was attributed to ingestion of rice contaminated with high levels of cadmium (Cd) (see Chapter 12).

In addition to heavy metals, a variety of inorganic and organic compounds can contaminate streams, lakes, and rivers, threatening their water quality. The observation that stream water or garden fertilizers may be contaminated with perchlorate is an example. Industrial and military operations and fireworks manufacturers use perchlorate as an oxidizing agent, and they appear to be the primary sources of contamination. Perchlorate is potentially harmful to thyroid function and could be widespread in some American agricultural areas because earlier studies by the EPA research laboratory showed that common garden fertilizers contained perchlorate concentrations up to 0.84% by weight. However, a subsequent study released by the agency showed that the majority of fertilizers used in the United States are not contaminated with perchlorate salts (Benner 2001).

Water pollution not only can influence human health directly but also can threaten aquatic life, particularly fish. For instance, in the early 1960s millions of fish in the lower Mississippi River died from the effects of chlorinated organic pesticides, particularly endrin. In the early 1970s, contamination of fish with DDT and PCBs (polychlorinated biphenyls) caused an abrupt halt to commercial salmon fishing in the upper Great Lakes. Although much progress has been made since, and the public is encouraged by the reports on the decreased levels of chlorinated hydrocarbons and other toxicants in fish crops, problems of water pollution in the Great Lakes appear to have persisted for some time (see Case Study 2.1).

Case Study 2.1

The Detroit News published a report, “Disappearing Shrimp Pose Threat to Great Lakes Whitefish.” According to the report, one of the principal food sources for whitefish is disappearing rapidly in the Great Lakes, a change that threatens to shake up the food chain and impede the state of Michigan’s large commercial fishing industry (Hirai 2010). The report shows that about 17,000 square miles in the Great Lakes no longer have diporeia, about a half-inch-long, shrimp-like organism. Research biologists indicated that they have never seen such a phenomenon before. In the 1980s, the scientists found densities of diporeia between 10,000 and 20,000 per square mile of sediment in parts of the Great Lakes. The researchers state that no diporeia are now found in many of the same spots. Diporeia is a main food source for many fish in the Great Lakes. Whitefish have become one of the first casualties of the loss of diporeia. Until recently, whitefish could be found that were about 2 feet long and 5 pounds. Now, whitefish range from 20 to 22 inches. The decline of the diporeia population remains somewhat of a mystery to fish researchers. They have examined whether the decline is a result of contaminants, but so far, there is no conclusive answer.

Case Study 2.2

Around the middle of 1960s, New York City’s Hudson River was found to be dying as a result of severe pollution. The sources of the pollution were found to be raw sewage

> being dumped into the river by the city; discharge of large quantities of paint from a plant; oil dumping from Penn Central Railroad; and discharge of water at elevated temperatures from a nuclear power plant.
>
> There is reason to be encouraged, however. In 1966, several fishermen formed the Hudson River Fishermen's Association. Mainly because of their effort and that of others who joined subsequently, much improvement was made. Beginning in 1968, a number of polluters were forced to spend millions of dollars remediating the Hudson. The by-product of these actions is considered one of the greatest environmental success stories of the century. Today, the Hudson produces more fish per acre than most other major estuaries of the North Atlantic. Fish and fishermen, boaters, and swimmers have reportedly returned to the river (Kennedy 1999).

A global census report published in 2004 showed that nearly a third of the world's amphibians were on the verge of extinction. Of the 5,743 known species of toads, frogs, salamanders, newts, and wormlike caecilians, 1,856, or 32.5%, were in danger of extinction, according to the Global Amphibian Agency (GAA), a joint effort by 500 researchers in 60 counties. Already, 122 amphibian species have disappeared since 1980, some apparently wiped out by illnesses fostered by warming temperatures; others were poisoned by pesticides. According to the GAA, since most amphibians depend on freshwater and feel the effects of pollution before many other forms of life, their rapid decline tells us that one of the earth's most critical life-support systems is breaking down (Attenborough 2008).

According to a 2005 report by the *International Herald Tribune* (Singapore), the Chinese government blamed China's biggest oil company, the China National Petroleum Corporation (known as CNPC), for a spill of an estimated 100 tons of benzene compounds into Songhua River. The spill was caused by an explosion of the plant, and state media reported that five people were killed in the explosion. An 80-km slick of the toxic compounds reached Harbin, the northern city of almost 4 million people on the river that normally supplies it with running water.

In Harbin, residents continued to stockpile bottled drinking water for several days after the authorities stopped pumping from the river to minimize the risk of poisoning. With the municipal water system shut down, schools and many businesses remained closed. The biggest environmental disasters forced the Chinese authorities to mount an investigation. China warned neighboring Russia about the toxic spill, which was being carried toward the border city of Khabarovsk, about 3,800 miles southeast of Moscow, and with 580,000 residents. The spill eventually reached the city, and these residents got water provided by city authorities.

The contamination of one of China's major rivers has drawn attention to the environmental price that the country is paying for an economic boom lasting three decades. Living standards have risen dramatically in many regions of China, particularly the provinces along the east coast, but severe environmental degradation has emerged as a threat to further development. According to news media, China's major cities are among the most polluted in the world, and vast tracts of farmland are being lost to erosion, industry, and desertification. But, it is the pollution of vital rivers, lakes, and groundwater in a country that is already short of water that looms as the biggest immediate threat, environmental experts indicate.

Even before the benzene spill, there were serious problems with water quality along the 1,850-km Songhua River, according to the Asian Development Bank (Lague 2005). The agency indicated in a July 19, 2005, statement that 62 million people lived in the Songhua River catchment area. The river, however, is one of the most polluted in the 47 major rivers in China. Contaminated with a number of organic chemicals, heavy metals, and other conventional pollutants, the river is considered unsuitable for municipal domestic water use. The bank was helping local authorities develop plans for pollution control along the river.

As if the benzene spill was not enough, a second human-made disaster hit a Chinese river 6 weeks later. According to an Associated Press news report, a dam temporarily blocked a toxic spill of cadmium from flowing downstream and reaching the country's southern business center, Guangzhow, a city of 7 million (*The Seattle Times* 2005). Authorities in southern China dumped water from reservoirs into the Bei River to dilute the cadmium spill.

According to news media, research showed that marine life in the Pearl River, China's third-longest river, contains excessive trace metals that may cause cancer. The research was started in 2003 and analyzed 58 marine samples collected in the Pearl River estuary in southern China; samples included fish, shrimp, and crab. The research showed that 1 kg of shrimp contained 0.835 mg of cadmium, more than 16 times the national standard. The content of lead in fish was 2.2 mg/kg, which also exceeds the national standard of 1 mg/kg.

Another large oil spill in northwest China heavily polluted a tributary of the Yellow River and threatened to reach one of the country's longest and most important sources of water. China's state-run news media said later that a "large amount of diesel oil had leaked out of pipeline in Shaanxi Province. The leak was caused by construction work, and a crew of 700 people struggled to contain the damage. The provincial government officials said that oil had been detected downstream from the leak and warned local residents not to use water in the region." The Yellow River, which stretches for about 5,500 km (about 3,400 miles), is a source of water for approximately 140 million residents, and it also provides water to factories and farms through northern China. As the spill threatened the Yellow River, residents were urged to find alternative sources of drinking water as authorities tried to stop the damage (*Time*, January 18, 2010, p. 18).

According to a report released by scientists at the U.S. Geological Survey (USGS), mercury (Hg) levels in the Pacific Ocean are expected to double relative to 1995 levels by 2050 if the emission rates of the metal continue as projected. The work showed for the first time that mercury originating from atmospheric emissions off the coast of Asia can be transported long distances by ocean currents. Previously, such long-range transport was thought to occur only in the air. The researchers sampled 16 sites in the eastern North Pacific Ocean and found that water samples collected in 2006 had 30% more mercury than samples collected in the mid-1990s. Although the scientists have yet to measure mercury levels in fish harvested from the Pacific to determine whether these levels also are on the rise, the National Fisheries Institute pointed out that "peer-reviewed research shows no mercury increase in oceangoing fish over the past 30 years" (*Science News* 2009). In response to the USGS study, the EPA administrator pledged to work with international partners to reduce mercury emissions from sources such as coal-fired power plants (USEPA 2010).

2.5 SOIL POLLUTION

Another major concern is the possible deleterious effect of the release of an increasing number of toxic synthetic chemicals into the environment. This leads to soil pollution, in addition to air and water pollution, and food contamination. Moreover, the release of these chemicals is not limited to areas adjacent to point sources such as industrial facilities. Rather, the chemicals can be transferred to distant areas and regions where they may elicit adverse effects on living organisms.

In the United States, an assessment of the extent and severity of contamination is further complicated by the nearly exponential growth of the synthetic organic chemistry industry since the early 1940s. Nearly 8,000 chemicals are estimated in common industrial and commercial use in the United States, and the number continues to grow every year. Only a limited number of ecological assessments on the bulk of the chemicals on the market or those introduced each year have been undertaken. The human health effect of many of these chemicals, particularly over long periods of time at low exposure levels, is largely undefined.

One of the widely known episodes related to disposal of hazardous wastes is that of Love Canal. It was an abandoned canal bed near Niagara Falls, in the state of New York. The episode is briefly discussed in Case Study 2.3.

Case Study 2.3

In the 1940s and 1950s, Hooker Chemical and Plastics Corporation dumped 23,000 tons of chemical wastes into the Love Canal landfill (Kirschner 1994). After the canal was filled and covered with earth, the land was transferred to the city of Niagara Falls. Homes and a school were then built on the edge of the old canal. The area of covered chemicals became a playground. In 1968, Occidental Chemical (OxyChem) purchased Hooker Chemical. In 1977, black oily fluids oozed from the ground in the vicinity of the canal. The fluids were subsequently identified as a mixture of potent chlorinated hydrocarbons. Children attending the school showed unusual health problems, such as skin rashes, chemical burns, and severe physiological and nervous disorders. Furthermore, an unusually high number of miscarriages and birth defects were noted. A lawsuit amounting to nearly $3 billion in health claims was then filed against the city of Niagara Falls. Eventually, the state purchased and demolished about 100 homes in the area, and state officials evacuated 500 houses in 1978. Federal and state crews cleaned up the landfill and surrounding contaminated areas. Litigation against each other followed between New York State and the company. In 1994, OxyChem and the state finally agreed to settle their conflicting claims stemming from the incident. (Note that remediation of the land eventually took place, and this was followed by resettlement of the area. By 1994, nearly 70% of the 280 available houses had been sold. A survey showed that about 30% of the purchasers were the residents in the area before the evacuation; Kirschner 1994.)

2.6 THE CHANGING DISEASES

Associated with the changes in our environment are the changing patterns and distribution of diseases. For instance, at the turn of the twentieth century, pneumonia and tuberculosis were the two leading causes of death in most countries. Because of improved sanitation and public health measures, together with advancing medicine and technology, tuberculosis and other contagious diseases have largely been eradicated. In place of these illnesses, however, are diseases that are more complex and have multiple causes. These include chronic heart diseases, chronic respiratory diseases, and cancer. It is widely known that, since about 1950, heart diseases and cancer have become the two leading causes of deaths in the United States. These two diseases as well as chronic lower respiratory diseases, chronic liver disease, and cirrhosis are considered environmentally related (U.S. Department of Health and Human Services [USDHHS] 2003, 2009) (Table 2.1).

Many diseases have long been known to be related to occupation. The British doctor Percivall Pott is widely recognized as the scientist who, in 1775, first pointed out the direct connection between an occupational exposure and the risk of a specific cancer (i.e., chimney sweeps and cancer of the scrotum) (Cole and Goldman 1975). Miners, stone cutters, and lens grinders often developed respiratory disease from inhaling large quantities of dust. Many hatters suffered brain damage as a result of absorbing highly toxic mercury vapors from mercurials (chemical compounds containing mercury) used in making felt. Asphalt, coal tar, and pitch workers; textile dyers; and shoe and leather workers are suspected of having an increased risk of developing bladder cancer because of their association with coal products and aromatic amines. However, in the past several decades, environmental diseases have spread beyond those in a few specialized occupations (Maltoni and Selikoff 1988). Several of the diseases are briefly discussed next. The discussion includes cancer, respiratory diseases, birth defects, heavy metal poisoning, and impact on reproductive systems. More detailed information is presented in subsequent chapters.

The changes in disease pattern have also been observed in many other countries, including the less-developed world. In Brazil, for example, in 1940 infectious diseases caused 39% to 60% of all deaths, depending on the region of the country. But, by 1980 these diseases accounted for only 3% to 16% of deaths. On the other hand, cardiovascular diseases accounted for only 9% to 13% of mortality in 1940 but rose to 20% to 38% in 1980 (Moran and Fleming-Moran 1996). Scientists consider that environmental pollution may play a role in such shifts. Many human diseases are traceable to substances in the air, water, and the foods we consume. Some of the industrial agents released into the general environment are known to be carcinogenic or suspected carcinogens. These are in the next section and subsequent chapters.

2.6.1 Cancer

The United States has one of the world's highest incidences of cancer associated with environmental pollution. "Exposure to environmental toxicants poses a serious threat to Americans, causing a grievous harm" that government agencies have

TABLE 2.1

Changing Causes of Death in the United States between 1950 and 2000

Year								
1950			1980			2005		
Rank		%	Rank		%	Rank		%
1	Disease of heart[a]	40.7[b]	1	Disease of heart[a]	39.6[b]	1	Disease of heart[a]	26.4[b]
2	Malignant neoplasm[b]	13.4	2	Malignant neoplasm[b]	23.0	2	Malignant neoplasm[b]	23.3
3	Cerebrovascular diseases	12.5	3	Cerebrovascular diseases	9.2	3	Cerebrovascular diseases	5.8
4	Unintentional injuries	5.4	4	Unintentional injuries	4.4	4	Chronic lower respiratory diseases[b]	5.4
5	Influenza and pneumonia (chronic nephritis)	3.3	5	Influenza and pneumonia	3.0	5	Unintentional injuries	4.9
6	Diabetes mellitus	1.6	6	Chronic lower respiratory diseases[b]	2.7	6	Diabetes mellitus	3.0
7	Suicide	0.9	7	Diabetes mellitus	1.7	7	Influenza and pneumonia	2.5
8	Chronic liver disease[b]	0.8	8	Chronic liver disease[b]	1.4	8	Suicide	1.3
9	Chronic lower respiratory diseases[b]	0.5	9	Suicide	1.1	9	Chronic liver disease[b]	1.1
10	Homicide	0.3	10	Homicide	1.0	10	Homicide	0.7

Source: USDHHS. Health, United States, 1996–97 and injury chartbook. 1997; USDHHS: Health, United States, 2009.

[a] Diseases that are considered environmentally related.

[b] Percentage of total deaths from all causes.

not adequately addressed, according to a strongly worded report released on May 6, 2010, by the President's Cancer Panel, a body of experts that reports directly to President Obama.

According to the American Cancer Society's estimates (ACS 2010), about 6% of cancer deaths—nearly 11,000 a year—are caused by environmental pollutants. Nearly 80,000 chemicals are used in the United States currently, many of which are not studied thoroughly and largely unregulated.

Since about 1950, cancer has been second only to heart diseases as the cause of death among the U.S. population. Moreover, until recently the rate of cancer deaths had been increasing steadily (Table 2.1) (Figure 2.3). Actual number of deaths from cancer, however, is still rising. For example, 416,509 Americans died of cancer in 1980. The figure increased to 505,322 in 1990 and to 549,838 in 1999 (USDHHS 2003). According to the American Cancer Society, the toll for 2003 was 556,500, and for 2006, it was 560,102—about 1,534 deaths a day (USDHHS 2009).

The northeast region of the United States is known as a highly industrialized and polluted area. This region is also known to be one with a particularly high incidence of cancer. According to the National Cancer Institute, areas where iron and lead smelters are located have high rates of lung cancer. Other studies showed that nearly 30% of the total mortality in several industrialized countries is due to cancer (*C&EN*, April 18, 1994, p. 13). Cancer incidence and mortality in most of these countries have been consistently increasing in recent decades. In particular, this trend is independent of the aging of the population.

Main types of cancer include brain/nervous system, female breast, colon and rectum, leukemia, liver, lung and bronchus, non-Hodgkin's lymphoma, ovary, pancreas, and prostate (USDHHS 2003). Among the suggested causes of cancer, environmental factors such as lifestyle, personal habits, diet, chemicals and radiation, and infectious diseases account for about three-quarters of all cancers. According to the American Cancer Society (2003), smoking, obesity, and physical inactivity have a greater effect on individual cancer risk than do exposure to trace amounts

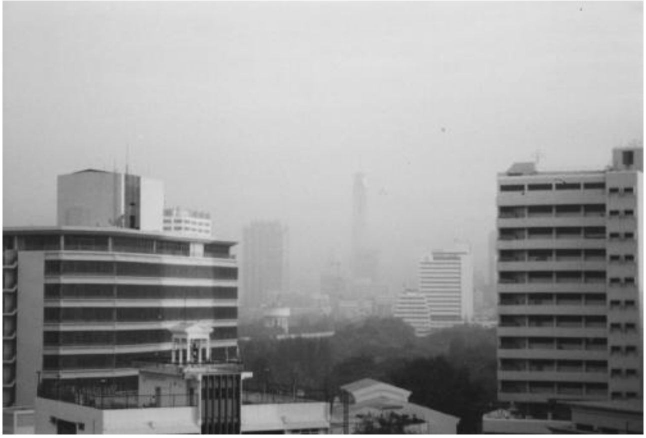

FIGURE 2.3 A smoggy day (August 2008) in Kuala Lumpur, Malaysia.

of pollutants in air, food, and drinking water. However, the degree of risk from pollutants depends on their concentration, exposure intensity, and duration. Evidence shows cancer risks increased in settings where workers were exposed to high levels of certain chemicals, such as heavy metals and organic compounds, as well as radiation. As noted previously, in the last 100 years, and particularly since World War II, following industrial development, a large number and quantity of chemicals have been released into the environment. The release had led to increased air, water, and soil pollution, potentially contaminating food. Areas with industrial plants manufacturing soaps, rubber, chemicals, and printing inks have high rates of bladder and liver cancer. Also, the New York Department of Health has found that Nassau County women living within 1 km of a chemical, petroleum, rubber, or plastics facility were 60% more likely to develop postmenopausal breast cancer than were those who lived in other parts of the country.

An alarming trend associated with cancer is the high incidence rate among children in the United States. According to the American Cancer Society, an estimated 1340 deaths were expected to occur among children aged 0 to 14 years in 2010 (American Cancer Society 2010). About 30% of the deaths could be from leukemia. Despite the rarity of childhood cancer in the United States, it is the second leading cause of death in children between ages 1 and 14 years, exceeded only by accidents (ACS 2010). The National Cancer Institute reported that the rate of increase has amounted to nearly 1% a year. Some experts in the field estimated that a newborn child today faces a risk of about 1 in 600 of contracting cancer by age 10 (ACS 2010). Although the reason for the high incidence rate of childhood cancer remains unclear, some scientists suspect that exposure of pregnant women and children to environmental pollutants may be an important factor. However, encouraging information was reported by the American Cancer Society (2003). It showed that the mortality rates of childhood cancer have declined by about 47% since 1975.

Cancer is especially lethal in young adults for many reasons. One is delays in diagnosis. Doctors seldom suspect cancer when young adults have symptoms like headaches, fatigue, or bone pain. Young people themselves often ignore symptoms, as well, so their cancers tend to be far advanced when they finally are detected. Furthermore, young adults are the least likely to have insurance, limiting the care they receive. And, few participate in clinical trials, so there is little data on the best treatments for them. A National Cancer Institute study showed that different types of cancers affect young adults aged 15 to 29 in the United States. The main types of cancer include lymphomas, invasive skin cancer, cancer of the male genital system, cancer of the endocrine system, cancer of the female genital system, leukemia, central nervous system cancer, and breast cancer (Table 2.2).

The study of the association of pesticides and related chemicals with various illnesses and death has attracted wide attention. Of particular concern are chlorinated hydrocarbon-based pesticides and dioxin. For instance, accidents during the manufacture of the herbicide 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) and polychlorinated phenol derivatives have caused acute dioxin poisoning of plant workers and populations in several countries.

As is widely known, in Vietnam 2,4,5-T and related dioxin-contaminated defoliants were used extensively from 1961 to 1969. Among the major toxic effects

TABLE 2.2
Types of Cancers that Affect Young Adults Ages 15 to 29 in the United States

Cancer Type	Percentage
Lymphomas	20
Invasive skin	15
Endocrine system	11
Male genital system	11
Female genital system	9
Leukemias	6
Central nervous system	6
Breast	5
Digestive system	4
Bone, joint	3
Soft tissue	3
Respiratory system	2
Urinary	2
Oral cavity	2
Other	1

attributed to dioxins is liver cancer. Between 1956 and 1961 (the year in which spraying of the herbicides began), 159 cases of primary hepatic cancers were recorded among 5,492 cancers in the Hanoi area, but between 1962 and 1968, 791 primary hepatic cancers were observed in a total of 7,911 cancers. This change represented a more than threefold increase in the proportion of primary cancer of the liver (Lapporte 1977).

A National Cancer Institute report indicated that farmers and people who work in their fields tend to have various kinds of cancer more often than the general population (National Cancer Institute 2011). More notable cancers include non-Hodgkin's lymphoma, brain cancer, and leukemia. Farmers also tend to be more prone to suffering from multiple myeloma and cancers of the brain, prostate, stomach, skin, and lip than the general population. To find out why, the federal government initiated a 10-year Agricultural Health Study with a budget of $15 million.

The National Academy of Sciences has reported that a lot more is known about the cancer risks and other health hazards from exposure to trichloroethylene (TCE) (Menon 2010) than there was in 2005 when the EPA took steps to regulate it more strictly.

TCE, which is also widely used to remove grease from metal parts in airplanes and to clean fuel lines at missile sites, is known to cause cancer in some laboratory animals. The EPA was blocked from elevating its assessment of the risks of the chemical in people by the Defense Department, Energy Department, and NASA, all of which have sites polluted with it. Its 379-page report recommended that the EPA revise the agency's assessment of the risks of TCE using "currently available data"—so no more time is wasted. That is a step that could lead to stricter regulations. EPA currently requires limiting TCE to no more than 5 ppb in drinking water.

Research into the major causes of death in adults in China found that the country has undergone a huge health transition. The research showed that over the past 45 years heart disease, cancer, and stroke have become the top killers of middle-aged people in China (*Cincinnati Post* 2005). The findings from the study of nearly 170,000 Chinese men and women over age 40 showed that about two-thirds of the 20,033 people who died during that time were killed by heart disease, cancer, or stroke. Chinese mortality rates from each of the three categories topped deaths among the same age group in the United States. According to China's Ministry of Health, pollution has made cancer the country's leading cause of death. Ambient air pollution alone is blamed for hundreds of thousands of deaths each year.

The conclusions were based on medical data collected in 1991 with follow-up evaluations in 1999 and 2000. Of these deaths involving people in their 40s to mid-60s, "We are very surprised by this finding," said lead coauthor Dr. He of Tulane University's Department of Epidemiology in New Orleans, Louisiana (He et al. 2002). "This study indicates that chronic disease is not only the leading cause of death in wealthy countries, but also in developing countries, such as China," he wrote. The results back up what Robert Beadlehole, the director of chronic diseases for WHO, has known for a long time. "I think it's probably exactly what it was like in the United States a couple decades ago," he said of China's health situation.

Lung cancer was the top cause of death in that disease category, and 63% of the men surveyed were smokers. High blood pressure was the top preventable contributing factor to the deaths, followed by cigarette smoking, physical inactivity, and being underweight.

The *New York Times* reported an unusual linkage between coal and cancer in China's Yunnan Province (*The New York Times*, November 14, 2010). Nonsmoking women in an area in the province died of lung cancer at a rate 20 times that of their counterparts in other regions of the country and anywhere else in the world. Subsequently, coal samples were analyzed by scientists at the University of Texas at Dallas. They found that quartz, of which silicon is the primary component, made up 13.5% of the coal samples taken from Xuanwei County of Yunnan Province. (In normal coal samples, quartz and other minerals are found only in trace amounts.) The high cancer rates in Xuanwei have attracted the attention of scientists for decades. Dr. Lan, an epidemiologist at the National Cancer Institute in Rockville, Maryland, is completing two studies involving hundreds of women and families there. While her team is confident that coal burning is causing the high rates of cancer, they are not certain it is due to silicon. A group of scientists now say that a possible explanation is the burning of coal formed during volcanic eruptions hundreds of millions of years ago. It was found that coal in that part of China contains high concentrations of silica, a suspected carcinogen. Like others in rural China, the families of Xuanwei County use coal for heat and for cooking. As the coal burns, silicon particles are released with the vapor and inhaled. Women, who do the cooking, face the greatest exposure.

2.6.2 Birth Defects and Child Mortality

It is estimated that approximately 3% of all live births in the United States have significant birth defects (Kalter and Warkany 1983). This represents about 100,000 congenital anomalies in a total of 3 million live births annually. Congenital malformations are the leading cause of infant mortality in the United States. Furthermore, studies showed that the presence of any malformation diagnosed during the first year after birth increased mortality 18-fold for white infants. Clearly, enormous financial costs and emotional suffering are associated with these malformations.

The etiologic nature of the majority of congenital malformations in infants is largely unknown. It has been estimated that about 5% to 10% of all birth defects are due to an in utero exposure to a known teratogenic agent (an agent capable of causing birth defects) or maternal factor. Intrauterine growth retardation can be caused by a number of agents, including hypoxia (a deficiency of oxygen reaching the tissues of the body), drugs, X-ray irradiation, maternal endocrine and nutritional factors, and environmental chemicals. Many chemical species are known to be teratogenic. These include various organic solvents, pesticides, dioxins, several heavy metals (e.g., lead, cadmium, mercury), and others. Many human epidemiological data support the claim that environmental chemicals are an important factor responsible for inducing teratogenicity.

Although child mortality rates have begun to drop, the United States lags in progress compared to many other countries. Underscoring historic recent gains in global health, the number of children younger than 5 who died in 2010 would fall to 7.7 million, down from 11.9 million two decades ago, according to new estimates by population health experts. But, as much of the world makes strides in reducing child mortality, the United States is increasingly lagging and now ranks 42nd globally, behind much of Europe as well as several less-developed countries. Twenty years ago, the United States ranked 29th in child mortality rate, according to data analyzed by the Institute for Health Metrics and Evaluation at the University of Washington. Singapore, the country with the lowest child mortality rate in the world at 2.5 deaths per 1,000 children, cut the rate by two-thirds between 1900 and 2010. The United States, in comparison, projected to have 6.7 deaths per 1,000 children in 2010, saw a 42% decline in child mortality, a pace on par with Sierra Leone and Angola.

2.6.3 Reproductive Damages

An increasing number of studies have shown that a variety of toxicants can induce detrimental effects on reproductive systems in animals and humans. For instance, reproductive damages in seagulls and other wildlife presented some of the first clues about the adverse effects of DDT. Organochlorines have also been implicated in impaired reproductive success in fish populations of the Baltic and North Sea (Barnthouse et al. 1990). These compounds also have detrimental effects on the health and reproduction of seals (Reijnders 1986).

More recently, reproductive anomalies in wildlife have sparked concern about the ability of a number of chemicals to cause ill effects by disrupting the body's normal hormonal system. An increasing number of chemicals are now known to have such action. Examples include organochlorines such as PCBs, dioxins, as well as DDT;

pesticides such as carbamates (e.g., aldicarb, carbofuran), triazines, pyrethroids; heavy metals such as cadmium, lead, and mercury; and organobrominate compounds.

The reproductive toxicity of the pesticide DBCP (2,2-dibromo-3-chloropropane) became clear in the late 1970s and early 1980s when male farm workers in the banana-growing region of Costa Rica were found to be sterile. By the mid-1990s, nearly 1,500 male workers had been diagnosed with sterility from exposure to DBCP (Thrupp 2001).

Studies showed a steady rise in premature births in the United States. According to U.S. government statistics, 11.8% of all babies or about 440,000 infants in 1999 were born prematurely—that is, before the end of the 37th week of gestation. (The normal length of gestation is 40 weeks.) According to the data from the National Center for Health Statistics (CDC 2010), 9.4% of live births were premature in 1981. Although strong evidence is still lacking, some researchers presented data at the Institute of Medicine-sponsored meeting in October 2001 suggesting that industrial chemicals, pesticides, and air pollutants could have contributed to the 23% rise in premature births in the United States since the early 1980s (Barrett 2002). One of the strongest associations was found in a study in which the levels of DDE—a metabolite of DDT—in stored sera of mothers who gave birth between 1929 and 1966, when DDT was heavily used in the United States. In a sample group of 2,380 babies born to these women, 361 were preterm, and 221 were small for gestational age. The greater the level of DDE in the mother's blood, the higher was the risk for the infant (WHO 1972).

Shortened gestation times were also reported to be associated with benzene exposure. A Chinese scientist studied 542 births to women working at a petrochemical plant in Beijing and found that benzene shortened the pregnancies of those women with a genetic profile that prevented them from detoxifying benzene easily (Hileman 2001).

2.6.4 Respiratory Diseases

Many epidemiological and animal studies have shown that airborne pollutants are commonly found in the urban environment in concentrations high enough to adversely affect the lungs (Hileman 2001). Since the 1960s, chronic bronchitis, emphysema, and lung cancer have become major public health problems in the United States and other industrialized countries. In the United States, although heart diseases have been known as the number 1 killer for several decades, and the death rates for the diseases increased between 1950 and 1960, they have since declined steadily. For instance, expressed as percentage of total death rate, the death rates of heart diseases for 1950, 1980, and 2005 were 40.7%, 39.6%, and 26.4%, respectively (Table 2.1). By contrast, the death rates for chronic lower respiratory diseases in the United States increased steadily until recently. For example, the rate for 1950 was only 0.5%, but it increased to 2.7% and 5.4% for 1980 and 2005, respectively (Table 2.1). A similar dramatic increase was observed with the respiratory system cancer death rate. For example, using the 1950 rate as the basis for comparison, the increases in cancer deaths from all causes were 7%, 46%, and 69% for 1960, 1980, and 2000, respectively. By contrast, the death rates for respiratory system cancer increased 80%, 490%, and 660% for 1960, 1980, and 2000, respectively (refer to Table 17.5). The marked differences

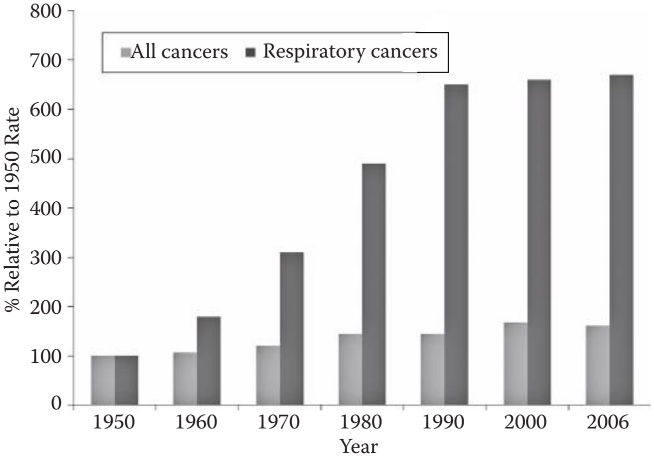

FIGURE 2.4 Comparison between death rates of all cancers and respiratory cancer in the United States.

in both categories of cancer death rates are more clearly shown in Figure 2.4. While the reasons for such differences are not entirely known, it is possible that exposure to increasing levels of air pollution may play an important role.

Smog, which is formed from chemicals emitted mostly by vehicles and power plants, is the nation's most widespread air-quality problem. According to the EPA, smog levels above 84 ppb threaten public health. Some studies found that even below that level, an increase of only 10 ppb leads to more deaths. Smog levels in many urban areas exceed 90 ppb on sunny summer days. The increase in deaths after a smoggy week is "relatively small," says Arden Pope, an air quality researcher at Brigham Young University in Provo, Utah. "The concern is that exposure is ubiquitous and the number of people affected is large. You take a small risk and spread it out over huge numbers of people, and you end up with a fairly large impact" (Pope 2007).

The study is the largest single investigation of the health effects of smog. It draws on data from 1987 through 2000. The results are likely to garner scrutiny from the EPA, which must decide in the next few years whether to hold counties to a more stringent smog goal than they now have (*NY Times* 2010).

In a study by Michelle Bell of Yale University, the strongest link yet between smog and damage to health was shown. The study, involving 95 U.S. urban areas, found that a small increase in the average smog level over 7 days can lead to a 0.5% rise in deaths, many from heart and lung disease, on the seventh day (Watson 2004).

The EPA announced in April 2004 that more than 450 of the nation's 3,141 counties do not meet the smog goal of the federal government. Those counties are home to nearly 160 million people—more than half the U.S. population (*USA Today*, 2004).

The other common form of dirty air that damages health is particle pollution, which exceeds federal standards in about 100 counties in the United States (Fennelly 1976).

In Japan, the level of air pollution has markedly decreased since the early 1970s, but the number of patients with respiratory disease due to air pollution was increased for a number of years until recently. Between the late 1950s and 1960s, many patients in Japan suffered from chronic obstructive lung diseases, such as chronic bronchitis, bronchial asthma, and emphysema. Studies showed that during that period, there were many patients with chronic bronchitis in Yokohama and Kawasaki, two highly industrialized cities near Tokyo that were heavily polluted with SO_2 and soot. Researchers in Japan concluded that the SO_2 pollution caused acute respiratory diseases and aggravated the conditions of patients suffering from respiratory disease. One of these respiratory conditions was even referred to as "Yokohama and Kawasaki asthma" (Murakami 1996).

2.6.5 Endocrine Disruption

Endocrine disrupters are chemicals that stimulate or retard the production of hormones. An increasing number of such chemicals have been shown to occur in the environment. They can cause adverse reproductive and developmental effects in humans and wildlife. Examples of endocrine disrupters include PCBs, bisphenol A, and some pesticides, such as DDT, methoxychlor, and lindane (Hileman 1999).

Although the impacts of these chemicals on humans and wildlife are complex, some aspects appear to be well accepted by scientists. For example, human prenatal exposure to PCBs, even at fairly low levels, can cause lower birth weight and shorter gestation; it has also been correlated with deficits in IQ and memory, as well as delayed neuromuscular development. More detailed discussion is presented in Chapter 15.

2.6.6 Diseases Induced by Metals

Following the Industrial Revolution, the production of various metals, such as copper, lead, and zinc, increased dramatically. Between 1850 and 1990, production of these three metals rose nearly 10-fold, with concomitant increases in the emission of other metals such as lead, cadmium, mercury, and nickel (Nriagu 1996). Another toxic chemical is arsenic. In limited geographic areas, some of these elements accumulate to excessive levels because of industrial activities and have produced major outbreaks of chronic illnesses in humans. Some highlights of these follow.

Although chronic lead poisoning has plagued humans at least since the time of the ancient empires, the importance of lead as an environmental pollutant has received widespread attention only in recent decades. In ancient Rome, lead in pipes and in drinking and cooking vessels was a major source of excessive intake. Even today, lead contamination in water supplies occurs in some communities. Lead pipes in older plumbing and soldered pipe joints can contaminate drinking water, especially "soft" water. However, the lead in smoke from burning trash and coal and, until recently, automobile exhausts is probably even more hazardous since it is inhaled or ingested as a contaminant of foods, after settling on vegetation.

Lead paint in older homes is even more dangerous because small children often ingest paint from woodwork, plaster, floors, and furniture. It is not surprising, therefore, that as many as 25% to 30% of American children living in urban areas may be suffering "subclinical" lead poisoning (Waldron 1974). The most prominent adverse effects of lead involve the nervous system, the hematopoietic system (an organic system of the body, consisting of the blood and the structures that function in its production), and the kidneys.

As mentioned, one of the most serious outbreaks of anthropogenic poisoning of the industrial age is the epidemic of mercury poising, now known as Minamata disease. This illness occurred in Minamata Bay, in Kyushu, Japan, in 1953, and the highest incidence was found to be among fishermen and their families (Kondo 1964). It was found later that household cats and seabirds were also affected. This turned the attention to fish and shellfish as etiologic factors. This in turn led to the study of the water of Minamata Bay and to the identification of mercury in a factory effluent as the cause of the disease. The study concluded that the fish consumed by victims contained high levels of toxic methylmercury (MeHg). When ingested, MeHg can induce permanent damage to the brain and kidney, loss of vision, and disturbed cerebral function. Ultimately, coma and death follow in severe cases.

The discovery of gold in Serra Pelada in the Amazon in 1979 touched off a great flow of migrants into that area in the 1980s. Many of these were in search of gold. Potentially serious health effects occur from high levels of metallic mercury exposure during gold mining. This is because mercury is used to bind the gold, and the resultant amalgam is heated at high temperatures with a blow torch to separate gold from the mercury. The mercury released to the environment gradually accumulated in the aquatic food chain. As a result, freshwater fish were contaminated with MeHg. In contrast to the mercury poisoning in Minamata, where a single industrial source polluted one local fishing area, in the Amazon region thousands of mercury sources pollute the waters. Brazilian mining agencies estimated that 300,000 miners had been distributed among 1,800 gold fields in the Amazon in the early 1990s. By 1996, some 3,000 tons of mercury had been released into the environment, compared to the 200 to 600 tons dumped into Minamata Bay. Many miners and residents were reportedly affected from the exposure to MeHg.

Another outbreak of chronic illness called itai-itai-byo or ouch-ouch disease occurred along the Jintsu River in northern Japan in the mid-1950s. Victims of this disorder suffered severe bone pains. Eventually, the softened bones of the victims' disintegrated even under slight pressure, resulting in multiple fractures. Death also occurred, and it was attributed to kidney failure that developed during the course of the disease. Extensive research ultimately identified the culprit as cadmium in rice grown near a lead and zinc mining facility (Aoshima 1999). Effluent from the mine used in irrigating rice paddies, combined with cadmium-laden fumes, had polluted the cultivated rice. In addition to its effect on bones, cadmium is a nephrotoxin, and it can cause hypertension. A more detailed discussion of heavy metals is presented in Chapter 12.

2.6.7 Foodborne Illnesses

According to U.S. Senator Richard Durbin (D-Ill.), more than 350,000 Americans are hospitalized each year, and 5,000 die from preventable foodborne illness (Weise 2010). He claimed that the United States needs to strengthen the state-federal partnership as we work to strengthen the nation's food safety laws. It is considered that state and local reporting of foodborne illnesses is the first line of defense against national outbreaks. A recent study by the Center for Science in the Public Interest found that almost half of states do a poor job of tracking outbreaks (*USA Today*, March 24, 2010).

An unusual food-related crisis occurred in China in 2008. The crisis occurred as a result of dairy products tainted with melamine, an industrial chemical. The incident caused kidney stones in 300,000 Chinese children and has been linked to the deaths of 6 infants. More than a year later, additional tainted dairy products were found in Chinese stores. Reportedly, in both incidents, the toxic nitrogen-rich chemical melamine (1,3,5-trizine-2,4,6-triamine) was intentionally added to products to boost the results of protein analyses. Health officials in southern China swept frozen confections and other dairy products from stores after discovering the products contained the chemical. It was the third time in a month that Chinese authorities had announced problems related to melamine, suggesting that producers were still making and selling tainted food ingredients despite outrage over the 2008 scandal and what the government heralded as a crackdown.

Another unusual incident of foodborne illnesses occurred in Japan in 2009. The illnesses were caused by ingestion of dumplings imported from China. A large number of people, including children, were intoxicated. Subsequent investigation revealed that the dumplings were contaminated with detergent. Shockingly, it was found by the owner of the Chinese restaurant that produced the dumplings that a female employee deliberately put the detergent into the dumplings.

REVIEW QUESTIONS

1. Briefly explain the air pollution episode that occurred in London in 1952.
2. What is air pollution? And, what are the main sources?
3. What are the six principal air pollutants?
4. Briefly describe the relationship between developing economies and environmental problems.
5. What is Minamata disease?
6. What does itai-itai-byo or ouch-ouch disease refer to?
7. Explain the water quality in New York City's Hudson River during the 1960s and the 1990s.
8. Briefly explain the Love Canal episode.
9. What is the most pronounced change in disease patterns in the United States between the turn of the century and 1950?
10. Name the five leading causes of death in the United States that are considered environmentally related.
11. What is the recent trend in the incidence rate of children's cancer in the United States?
12. What does *teratogenic* mean?
13. Briefly explain how environmental chemicals may be associated with the reproductive system.
14. Explain the differences between the total cancer death rates and the respiratory system cancer death rates in the United States between 1950 and 2006.

15. What are the most prominent adverse effects of lead poisoning?
16. What environmental problem exists in gold mining in the Amazon Basin?

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3 Occurrence of Toxicants

3.1 INTRODUCTION

A variety of pollutants is found in our environment. They arise from many sources, and exposure to them can occur through different routes. For example, in the ambient air in urban areas, SO_2, CO, NO_x, and smoke, suspended particles, lead (Pb), and hydrocarbons are produced largely from coal or heavy oil combustion by industries, power plants, and some households. A number of pollutants are also found in the indoor environment. Some examples are CO arising from incomplete combustion of fossil fuels and tobacco smoke, lead from paint used in old houses, and formaldehyde from insulation and wood preservatives and adhesives. In this chapter, where and how certain toxicants may occur in our environment are discussed. Also included in our discussion is a brief review of a number of pollution episodes or disasters that took place in recent decades.

3.2 VISIBLE SMOKE OR SMOG

The presence of visible smoke or smog is a manifestation of air pollution. Smoke is the gaseous product of burning carbonaceous materials made visible by the presence of small particles of carbon. The brownish-to-blackish materials emitted from the stack of an inadequately controlled coal-burning industrial plant or from the chimney of a wood-burning home are examples. Wood burning has become a common practice in many American homes, especially in winter. Burning wood in a well-insulated home, however, can lead to indoor pollution. The problem associated with indoor air pollution is particularly serious in many villages in southern China, where indoor combustion of coal as a means of cooking meals or drying vegetables is commonly practiced.

Smog, on the other hand, is a fog made heavier and darker by smoke and chemical fumes. Smog is formed mainly as a result of photochemical reactions. In the presence of ultraviolet rays in sunlight, nitrogen dioxide (NO_2) is broken down into nitric oxide (NO) and atomic oxygen. Atomic oxygen can then react with molecular oxygen in the air to form ozone (O_3). In addition, a large number of chemical reactions occur among hydrocarbons or between hydrocarbons and NO, NO_2, O_3, or other chemical substances in the atmosphere, resulting in the formation of numerous chemical species. Both NO and NO_2 are called *primary air pollutants* as they are formed at the source. On the other hand, O_3 and other compounds produced from chemical reactions that occur after the primary pollutants are emitted into the atmosphere are called *secondary pollutants*. Ozone, PAN (peroxyacyl nitrate), and some aldehydes and ketones are examples. NO_2 can be included as a secondary pollutant as well.

Smog is composed of both primary and secondary air pollutants because it contains NO_2, O_3, and other photochemical oxidants and a large number of other chemical species. Although Los Angeles is widely known for its smog, many large cities are suffering increasingly from similar problems. For example, Mexico City, with an estimated population of more than 20 million, has long experienced serious air pollution problems, although some improvement has been made recently.

Both smoke and smog cause visibility reduction because light is scattered from the surfaces of airborne particles. They cause deleterious effects on vegetation, animals, and humans.

3.3 OFFENSIVE ODORS

Malodors are often the first manifestation of air pollution. They are present in natural air, households, farms, sewage treatment plants, solid waste disposal sites, and many industrial areas. Natural air may contain odors arising from different sources. Decomposition of organic matter containing proteins derived from vegetation and animal life could contribute to odors in the air.

Odors from cooking foods such as fish, meat, and poultry in the kitchen can contribute to the odors sensed in a household. Fresh paints, fresh carpets, furniture polish, cleaning fluid, wood-burning fireplaces, and deodorants are some other examples. Cigarette smoking in a household and in a public place can also be an important cause of odors. Most public places are now smoking free, however.

Offensive odors may be detected in areas adjacent to industries, and they vary with the type of industries involved. Some examples include the so-called rotten egg type of odors in the air surrounding some pulp mills due to the presence of hydrogen sulfide (H_2S); odors from oil refineries due to H_2S and mercaptans (RSH); odors from some chemical plants due mainly to aniline or organic solvents used; odors from food-processing plants; odors from iron and metal smelters emitting acidic smells; and those from phosphate fertilizer manufacturing plants.

3.4 AGRICULTURAL DAMAGE

Agricultural damage constitutes the major damage by air pollution to vegetation (to be discussed in more detail in Chapter 8). A widely known example is the destruction of forests by acid rain. Many reports exist attesting to this phenomenon in the United States, Canada, and some European countries. Acid rain causes changes in plant growth that are manifested by stunted growth, lack of vigor, reduced productivity, and early senescence of leaves. Air pollutants such as SO_2, NO_2, O_3, PAN, and fluoride can also cause serious injuries to plants. Many fruit trees and vegetables are particularly sensitive to these pollutants.

Assessment of the immediate and long-term economic effects of air pollution on agriculture has been difficult because of the many variables involved. However, available information indicates that the cost due to decreased crop yields is staggering. Losses to producers from O_3 alone were estimated at $1 billion to $5 billion in 1986 (Cerceo 1987). An estimated cost of damage caused by acid rain to 32 major crops in the United States was $50 billion.

Injuries to plants by air pollution are often manifested by such symptoms as chlorosis and necrosis. Chlorosis is the fading of natural green color, or yellowing, of plant leaves. It occurs as a result of the destruction of chlorophyll or interference with chlorophyll biosynthesis. Necrosis, on the other hand, refers to localized or general death of plant leaves. It is often characterized by brownish or black discoloration of the leaf.

3.5 INTOXICATION OF ANIMALS

Reports of the injuries of fish and wildlife caused by water pollution abound in the world. In the United States, it has been estimated that more than 1 million waterfowls are killed every year due to ingestion of spent lead pellets left after hunting. A number of sea mammals were washed ashore in different parts of the world in recent years, apparently dead due to altered immune systems following exposure to waterborne toxicants.

A new type of environmental disease has also appeared and drew the attention of many scientists. Biologists have noted dramatic declines in amphibian populations since about 1991 and increases in frog deformities with no apparent cause in remote, high-altitude areas of the western United States, Puerto Rico, Costa Rica, Panama, Colombia, and Australia. The declines represented a sharp departure from previous years, when amphibian populations had crashed only from habitat destruction or the introduction of exotic predator species. Scientists fear that many species of amphibians that have been on Earth for 350 million years will not survive the twenty-first century. They view these population losses as an indication that there may be something seriously wrong with the environment. Suggested possible causes include infections and the effects of synthetic organic compounds such as pesticides, metallic contaminants, acid precipitation, UV radiation, and global warming. But so far, there is no conclusive evidence that any of these is responsible for the declines. Many scientists believe that several factors may be acting synergistically to produce the rapid die-offs (Hileman 1998).

Researchers from Conservation International and the Amphibian Specialist Group of the International Union for the Conservation of Nature have launched an unprecedented search of the planet's remotest spots to try to find 100 "lost" amphibians, ones feared extinct but that may be holding on in a few isolated places. According to the teams of scientists, many of the amphibians that they were looking for had not been seen in several decades, in some cases nearly 100 years. It was considered important to establish whether populations have survived or not to understand the recent amphibian extinction crisis. As mentioned in Chapter 2, the tiny Kihansi spray toad, which once numbered at least 17,000 at the Kihansi Falls in Tanzania, has been shown to be extinct in the wild.

It is known that the amphibians also provide many important services to humans, such as controlling insects that spread diseases and damage crops and helping to maintain healthy freshwater systems. The chemicals in amphibian skins are also important in helping to create new drugs with the potential to save lives.

3.6 INJURIES TO HUMANS

Many people in different countries have suffered injuries through exposure to high levels of air- or waterborne pollutants. For instance, exposure to high levels of air pollutants can cause various unfavorable physiological changes. Air pollutants such as SO_2, O_3 and other oxidants, and particulate matter have been regarded as responsible for causing pulmonary disease, heart failure, coughing, or degeneration of the lining of the throat. Some of the injuries are fatal, while others may induce permanent disability. Historically, such human injuries occurred only in certain occupations, but in recent years events leading to injuries or death have occurred as a result of non-occupation-related factors.

According to studies, the prevalence of asthma among children and young adults has increased in recent decades. This trend persists today, mostly in affluent countries (Thomas 1997). In many countries where asthma is common, its occurrence has jumped nearly 50% in 10 years. Furthermore, hospitalization rates in these countries have also risen, and deaths attributed to asthma have sharply increased as well. For example, asthma mortality among persons 5 to 34 years of age rose more than 40% between the mid-1970s and mid-1980s in most countries studied (Sears 1991). While the reason for this trend has not been established, many scientists consider that it is associated with environmental toxicants.

Individuals exposed to various environmental toxicants may suffer from different signs and symptoms without knowing the cause at the time of exposure. Furthermore, symptoms may not be manifested immediately following exposure. For example, many of the shipyard workers who were exposed to asbestos during the 1940s were not diagnosed until 15 to 30 years later. Other examples include Minamata disease and the itai-itai byo mentioned in Chapter 2, and the *Yu-sho* or “oil disease” that occurred in Japan as a result of consumption of rice oil highly contaminated with polychlorinated biphenyls (PCBs).

Human exposure to pesticides can occur directly, especially for agricultural workers and their families and those residents living in areas adjacent to farms where pesticides are heavily used. Indirect exposure also occurs when pesticide residues on food or contaminated fish are ingested. Some synthetic organic pesticides are slow to degrade, and they can persist in the environment for years. These chemicals can accumulate in human tissues and induce health problems.

It is clear that an enormous effort has been made by the U.S. government, industries, and the public to reduce environmental pollution. Such effort has led to encouraging results. According to the 1994 annual assessment of urban air pollution by the U.S. Environmental Protection Agency (EPA), the quality of U.S. air has been improving. Nevertheless, 43 metropolitan regions where nearly 100 million Americans live had O_3 levels higher than 0.12 ppm, exceeding federal health standards. The air pollution problem in the Los Angeles basin is known to be particularly serious; many environmental scientists consider it the most polluted city in the United States. In addition, five other cities in California are included in the 10 most polluted cities in the United States, according to a recently published *Forbes* magazine article (Chris 2011).

3.7 CHRONIC AND ACUTE EFFECTS

In studying the health effects of toxicants on living organisms, researchers often identify them as *chronic* or *acute* effects. A chronic effect is characterized by a long-term or recurrent exposure to relatively low concentrations of toxicants. Signs and symptoms differ depending on the types of toxicants, their concentrations, and species of exposed organisms. An acute effect, on the other hand, refers to that manifested by severe injuries or even death of an organism. It is characterized by exposure to high concentrations of a toxicant for a short period of time.

3.7.1 CHRONIC EFFECTS

Chronic intoxication is more common than acute episodes of poisoning. Numerous reports have shown the chronic effects of both air and water pollution on living systems. Long-term exposure to relatively low concentrations of air pollutants such as SO_2, smoke, and heavy metals such as lead, cadmium (Cd), and mercury (Hg) may eventually induce injuries to plants, animals, or humans. The Minamata Bay incident and the itai-itai byo mentioned previously are examples of chronic effects related to water pollution. Some of these are discussed in more detail in subsequent chapters.

In plants, chronic effects are manifested in impaired growth and development, decreased respiration, chlorosis, necrosis, and other symptoms. Similarly, in animals, chronic effects are reflected in retarded growth, increased susceptibility to other environmental stresses, and shorter life spans.

In humans, the health effects of exposure to air pollutants may occur over a long period of time. A prolonged exposure to air pollutants such as NO_2 and O_3, for instance, may lead to chronic bronchitis and emphysema. In the United Kingdom, the combination of SO_2 and smoke pollution is thought to have synergistic effects with cigarette smoking, resulting in degenerative diseases (Goldsmith and Friberg 1977). Results from occupational studies strongly suggest a close association between air pollution exposure and respiratory cancer. For example, inhalation of toxic materials, such as arsenic, asbestos, chromium, soot, mustard gas, and radon, under occupational conditions, has been related to lung cancer (Xu et al. 1995).

In an effort to study the association between air pollution and daily outpatient hospital visits, Wong et al. (1996) collected data on nonsurgery outpatient visits at a community-based hospital in Beijing, China, and on atmospheric SO_2 and total suspended particle (TSP) levels. Analysis of the data showed increases of 20% and 17% in nonsurgery outpatients to the hospital in association with increases in SO_2 and TSP levels, respectively. These observations led to the conclusion that the existing air pollution levels in Beijing were associated with adverse health effects.

A similar observation was made in Hong Kong, in which Wong et al. (2001) studied the levels of SO_2, NO_2, O_3, and atmospheric particulate matter less than 10 μm in diameter (PM_{10}). Their studies showed a significant association between cardiovascular and respiratory diseases and daily hospital admissions, both combined and separately. Furthermore, the effects of the pollutants on circulatory and respiratory diseases were shown to be stronger for older age groups, with significant excess of 5% to 10% in those aged 65 and over. Both NO_2 and O_3 were strongly associated with hospital deaths from cardiovascular and respiratory diseases (Mehta et al. 1990).

3.7.2 ACUTE EFFECTS

Since 1930, a number of acute pollution episodes have occurred in different parts of the world. Several of these episodes are reviewed here.

3.7.2.1 Donora, Pennsylvania, United States, 1948

On October 26, 1948, thermal inversion and foggy weather caused 20 deaths, and nearly half of the population of 14,000 of Donora, Pennsylvania, became ill with severe coughing. Many industries, including a large steel mill, a zinc production plant, a sulfuric acid plant, and other industries were located in this small industrial city. High levels of SO_2 and particulate matter were suspected as the cause of the suffering.

3.7.2.2 Poza Rica, Mexico, 1950

An incident occurred on the early morning of November 24, 1950, and was caused by the accidental release of H_2S from a natural gas plant in the city of Poza Rica, Mexico. At the time of the accident, most of the nearby residents were still in bed or had just arisen. Many residents were quickly affected with respiratory distress and central nervous systems damage. Twenty-two people died as a result, and more than 300 were hospitalized.

3.7.2.3 London, England, 1952

This is the most widely known air pollution episode. It occurred in London during December 5 through 8, 1952, and was the result of fog and thermal inversion. Many people suffered from shortness of breath. Cyanosis, some fever, and excess fluid in the lungs were reported in many patients. High levels of SO_2, fluoride, and smoke were recorded in the air. According to municipal statistics, about 4,000 excess deaths occurred. The figure obtained was the difference between the average deaths from 1947 to 1951 and the number of deaths that occurred during the episode (Figure 3.1). Most of those affected were in the older age groups and generally had disease of the heart or lungs prior to the pollution episode.

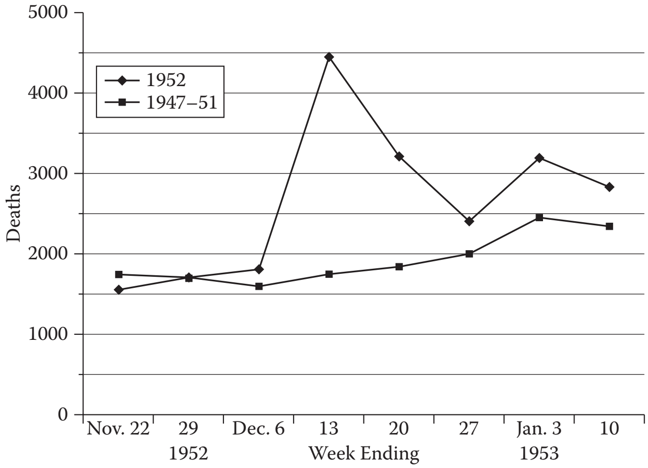

FIGURE 3.1 Excessive deaths in London, England, during the air pollution episode of December 5–8, 1952.

3.7.2.4 New York, United States, 1953

This episode occurred from November 18 to 22, 1953, in New York City and was the result of air stagnation and the presence of a high level of SO_2. The episode resulted in several thousand excess deaths.

3.7.2.5 Los Angeles, California, United States, 1954

Unlike those episodes mentioned previously, the cause of this episode was smog formation and the accumulation of high levels of photochemical oxidants, such as O_3 and PAN. Excess deaths totaling 247 per day in the 65- to 70-year age group were among the observed casualties.

3.7.2.6 New Orleans, Louisiana, United States, 1955

This 1955 episode in New Orleans, Lousiana, was marked by a sharp increase in the incidence of asthma among the residents of the city. The normal frequency of visits to a local hospital was reported to be an average of 25 per day, but during the episode period, it was 200 per day. The suspected cause was dust from flour mills.

3.7.2.7 Worldwide Episode, 1962

This air pollution episode lasted from November 27 to December 10, 1962, and involved London, England; Rotterdam, the Netherlands; Osaka, Japan; Frankfurt, Germany; Paris, France; Prague, Czechoslovakia; and the eastern part of the United States. Patients in the United States suffered upper respiratory symptoms. There were 700 excess deaths in London and 60 in Osaka.

3.7.2.8 Tokyo, Japan, 1970

This episode occurred in Tokyo, Japan, on July 18, 1970, and was due to high levels of oxidants and SO_2 in the atmosphere. More than 6,000 people complained of severe eye irritation and sore throat. One of the authors of this volume (Yu) visited Tokyo in 1972. He saw a large area of the city covered with smog, and the famous Tokyo Tower was barely visible. (He has visited the city several times since and has been impressed with the generally favorable air quality, particularly considering that the city's population is about 20 million.)

3.7.2.9 Bhopal, India, 1984

On the morning of December 3, 1984, an extremely serious industrial accident occurred in Bhopal, India (Figure 3.2). Forty tons of the highly toxic gas methyl isocyanate (MIC) (CH_3–N=C=O) leaked from Union Carbide's pesticide plant located in Bhopal and diffused into densely populated adjacent neighborhoods. Reportedly, between 8,000 and 10,000 people were killed in the days following the accident. It resulted in the deaths of a total of 25,000 people, and 100,000 people continue to suffer from chronic illnesses stemming from gas exposure (Brown 1994). It was observed that lungs were the main target organ of MIC. A hospital report released 3 days after the incident showed occurrence of alveolar and interstitial edema and emphysema among the victims treated. The large number of deaths and injuries,

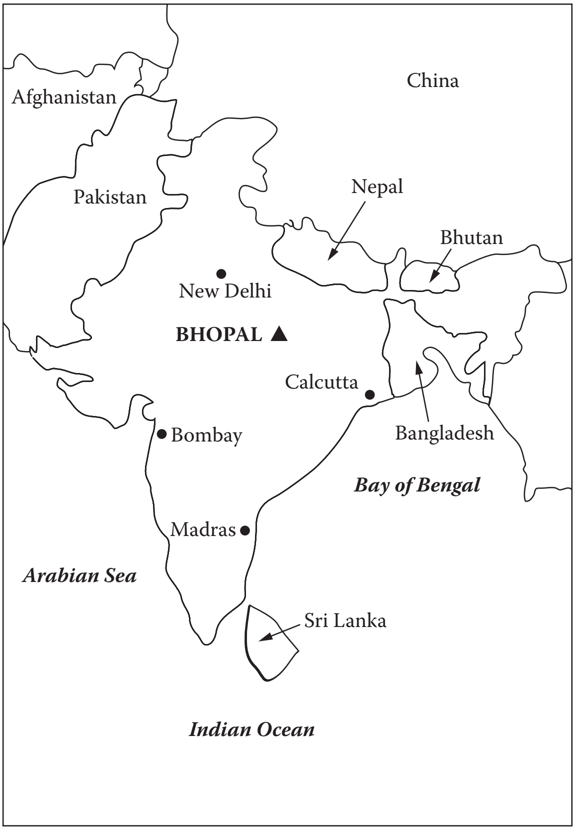
China
Afghanistan
Pakistan
Nepal
Bhutan
New Delhi
BHOPAL
Calcutta
Bombay
Bangladesh
Bay of Bengal
Madras
Arabian Sea
Sri Lanka
Indian Ocean

FIGURE 3.2 Location of Bhopal in India.

many permanently disabling, made the accident the greatest acute chemical disaster ever (*Wall Street Journal* 2010).

The tragedy in Bhopal brought chilly attention throughout the world. In June 2010, a district court in Bhopal found seven former officials of Union Carbide India Limited "guilty of causing death by negligence" in the gas leak there 25 years prior (Fastenberg 2010).

3.7.2.10 Chernobyl, Soviet Union, 1986

By far the gravest disaster in the history of commercial atomic power occurred on April 26, 1986, at Chernobyl in Ukraine, then a state of the Soviet Union (Figure 3.3). The number 4 reactor of the Chernobyl Nuclear Power Station partly melted down and exploded, killing 32 people in the immediate area and causing 237 cases of acute radiation sickness (Fastenberg 2010). Furthermore, the explosion sent a devastating cloud of radiation across a wide swath of Europe. Radioactive forms of iodine, cesium, strontium, and plutonium were released into the atmosphere and deposited throughout the Northern Hemisphere. The 30-km zone surrounding the station, from which 115,000 people were evacuated, received especially high exposure; the risk

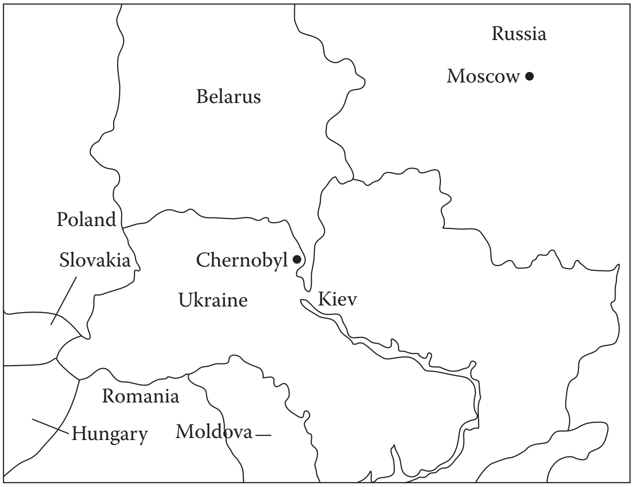

FIGURE 3.3 Location of Chernobyl.

of spontaneous leukemia was estimated to be double for these people for the next decade, and some genetic disorders may appear in individuals who were exposed in utero (Anspauch et al. 1988). The total radioactivity of the material released from the reactor was estimated to be 200 times that of the combined releases from the atomic bombs dropped on Hiroshima and Nagasaki according to a 1995 World Health Organization (WHO) report (Mershon 2011).

The accident exposed millions of people, notably in Belarus, Russia, and the Ukraine, to varying doses of radiation. According to the Organization for Economic Cooperation and Development Nuclear Energy Agency (OECD/NEA), 20 radionuclides were released into the atmosphere. They included iodine-131 with a half-life of 8 days; cesium-134 and cesium-137 with half-lives of 2 and 30 years, respectively; and several plutonium isotopes with half-lives ranging from 13 to 24,000 years. Subsequent studies indicated a dramatic increase in the incidence of thyroid cancer in children, mainly in Belarus and the Ukraine and to a lesser extent in Russia (Freemantle 1996).

3.7.2.11 Gas Leak on the Platform in the North Sea, 1988

In 1988, a gas leak on the platform of Piper Alpha, located off Scotland in the North Sea, caused an explosion, killing 167 workers. It was considered the world's worst offshore drilling disaster (Christen 1999).

3.7.2.12 Oil Spill in Alaska's Prince William Sound, 1989

Crude oil from the North Slope fields in Alaska is carried by pipeline to the port of Valdez and then shipped by tanker to the West Coast. On March 24, 1989, a huge tanker named *Exxon Valdez* went off course in a 16-km wide channel in Prince William Sound near Valdez, a harbor town of 4,200. The tanker struck a reef, causing the worst oil spill ever in U.S. waters. Eleven million gallons of crude oil escaped and coated more than 2,000 km of shoreline, killing an estimated 250,000 seabirds, 2,800 sea otters, 300 harbor seals, 250 bald eagles, as many as 22 killer whales, and countless other marine mammals and fish. The spill also injured an unknown number of salmon and herring eggs and larvae (Short et al. 2004).

While many scientists believed that the affected areas had recovered within a decade after the disaster, a new study by Short et al. (2004) at the Alaska Fisheries Science Center, National Oceanic and Atmospheric Administration (NOAA), made an alarming observation. According to the study, oil was found on 78 of 91 beaches randomly selected. The cumulative area of beach contaminated by surface or subsurface oil was estimated at 11.3 ha, and the mass of remaining subsurface oil was about 0.2% of the original oil. The results of their study suggest that the toxicity stemming from the oil, primarily from polyaromatic hydrocarbons (PAHs), continues to affect the recovery of some sea animals in places where the oil is most persistent.

Even in 2009, at 20 years after the spill, residual oil could still be found in sediment along Prince William Sound beaches. The biggest lingering concern from the disaster, however, is whether it could happen again. Rick Steiner, a professor at the University of Alaska, indicated that, even with the safety system in Prince William Sound, things can still go wrong. His remarks were substantiated by several close calls since the spill. They include those that occurred in September 20, 1989; July 30, 2001; October 10, 2002; and March 13, 2005 (*USA Today* 2010).

On the other hand, some scientists consider that the *Exxon Valdez* disaster was revolutionary. They pointed out, for instance, that the disaster spawned sweeping safety upgrades in the sound, including more tug escorts for tankers, improved radar monitoring of ship traffic, and a greatly expanded arsenal of spill containment and cleanup equipment, as well as initiation of cleanup drills.

The *Exxon Valdez* disaster also produced a federal law that all large tankers have a double hull by 2015, which could have greatly reduced the spill. In addition, the people transporting oil have, since the Exxon disaster, become more environmentally aware (Loy 2009).

3.7.2.13 Coal Mine Explosion in Western Virginia, United States, 2010

Twenty-nine workers were killed as a result of a coal mine explosion in western Virginia, United States on April 8, 2010. The explosion was caused by high levels of CH_4 gas mixed with coal dust. The death toll made it the worst U.S. mining disaster since a 1970 explosion that killed 38 people in Hyden, Kentucky. The deadly mine explosion prompted President Obama to order a review of the nation's troubled coal mines (*USA Today* 2010).

3.7.1.14 Gulf of Mexico Oil Spill, United States, 2010

On April 20, 2010, an explosion rocked the Deepwater Horizon oil rig in the Gulf of Mexico, killing 11 workers onboard. The drilling rig burned for more than a day before sinking into the sea. The well spewed millions of gallons in the nation's biggest oil spill since the *Exxon Valdez* disaster in Alaska. Within days, the spill had produced an oil sheen more than 1,800 square miles large, threatening the sensitive Gulf coastline (*USA Today* 2010). It was the worst offshore oil spill in U.S. history.

Subsequent studies by government scientists showed that the leak amounted to 500,000 to 800,000 gallons per day (www.cen.online 2010). The toxic effects of an oil spill on exposed oceanic organisms are complex. Some researchers show that when organisms absorb or even eat small amounts of oil over a period of time, their cells divert energy from growth and reproduction to defending themselves from the toxic oil. As a result, later in these organisms' life cycles, they develop growth defects that limit their viability. Also because oil's toxic aromatic compounds act like narcotics or anesthetics, nonlethal doses can slow the growth of fish and disrupt their ability to respond to predators or to catch prey (Johnson and Torrice 2010).

The economic impact caused by the oil spill is huge. For instance, toward the end of July 2010, the oil giant announced that it would set aside $32.2 billion to cover the long-term costs of the spill (*Time* 2010).

3.7.2.15 Raspadskaya Coal Mine Explosion in Russia, 2010

On May 8, 2010, two explosions occurred in Russia's largest underground coal mine, Raspadskaya, located in Siberia's Kemerovo region. Sixty workers were killed, and more than 30 were trapped up to 488 m (1,600 ft) below the surface. The cause of the blast was presumed to be a buildup of CH_4 gas.

It was reported as the worst Russian mining disaster since 2007, when a CH_4 explosion in the same region killed 110 workers (nytimes.com 2010).

3.7.2.16 Gas Explosion and Chemical Leak in Nanjing, China, 2010

A deadly gas explosion and a chemical leak occurred on July 28, 2010, killing at least 12 and injuring scores outside a plastic plant in Nanjing, China. It is reported that mishaps that threaten lives and the environment are an everyday occurrence in China's workplaces, such as in coal mines, fireworks plants, or at ports. This is considered another example of problems with China's industrial safety. According to a recent Chinese government report, an average of 187 workers die daily in mishaps, as focus on growth often trumps safety concerns. Scientists consider that Beijing's decades-long emphasis on economic growth has overshadowed efforts to ensure safe workplaces and a clean environment. Also compounding the risks are patchy zoning rules in Chinese cities, which sometime mean homes closely surround factories. For example, the 66-building compound of the plastics factory, where the gas explosion occurred, sits in an industrial-residential area, near a furniture shop and doctor's office, not far from provincial government offices downtown and the city's major park (Areddy 2010).

3.7.2.17 Toxic-Sludge Spill in Hungary, 2010

The wall of a huge sludge waste reservoir from a nearby aluminum processing plant in Kolontar, in southern Hungary, broke, and toxic sludge poured from a broken containment pond on October 6, 2010. The sludge flooded through a series of villages, bursting into homes and overturning vehicles.

According to the government, the accident, apparently caused by human error, resulted in 7 deaths and more than 100 injuries. It was estimated that 600,000 to 700,000 m^3 of toxic sludge spilled from the reservoir and polluted an area of 15 square miles, including tributaries of the Danube (*Wall Street Journal,* October 7, 2010; nytimes.com, October 8, 2010).

3.7.2.18 Gas Explosion in Henan, China, 2010

Another 37 coal miners died in Yuzhou City in central Henan Province in China on October 16, 2010, following a gas explosion. A separate blast occurred in 2008 in the same pit, killing 23 miners. According to the state-run Xinhua News Agency in China, China suffers the highest absolute number of coal-mining-related deaths—in 2009, there were 2,631 deaths (Wong 2010).

By a total coincidence, during the same month, 33 miners in Chile were dramatically rescued after being confined underneath a mine for 2 months following a gas explosion. (Luckily, no one was killed.) China is the world's largest producer and consumer of coal, and the Chilean mine rescue shed light in China. According to a report by the China News Service, Beijing's top mine safety official promised change and faster installation of underground shelters and other emergency facilities (www.usatoday.com 2010).

3.7.2.19 Fukushima Nuclear Power Plant, Japan, 2011

On March 11, 2011 an earthquake categorized as 9.0 Mw on the moment magnitude scale occurred off the northeast coast of Japan. At the Fukushima Nuclear Power Plant, units, 4, 5, and 6 had been shut down prior to the earthquake for planned maintenance. The other reactors were shut down automatically after the earthquake, and the remaining decay heat of the fuel was being cooled with power from emergency generators (Wikipedia 2011). The subsequent destructive tsunami with waves up to 14 m (the reactors were designed to handle up to 6 m) disabled emergency generators required to cool the reactors. Over the following 3 weeks, there was evidence of partial nuclear meltdowns in units 1, 2, and 3; visible explosions, suspected to be caused by hydrogen gas, in units 1 and 3; a suspected explosion in unit 2 that may have damaged the primary containment vessel; and a possible uncovering of the units 1, 3, and 4 spent fuel pools. Radiation releases caused large evacuations, and there were concerns about food and water supplies and treatment of nuclear workers.

The events at units 1, 2, and 3 have been rated at Level 7 (major release of radioactive material with widespread health and environmental effects requiring implementation of planned and extended countermeasures) on the International Nuclear Event Scale, and those at unit 4 as Level 3 (Serious Incident) events. On April 3, two bodies were discovered in the basement turbine room after the workers likely ran there during the tsunami (Wikipedia 2011).

Within days of the disaster, at the Fukushima Daiichi nuclear plant, Japanese food inspectors were spot-checking meat from the region's slaughtered cattle for radioactive contamination (Dvorak and Osawa 2011). Officials later fanned out to farms and near the crippled plant to pass Geiger counters over the animals to determine whether they were safe to sell. On March 31, Agriculture Ministry officials assured consumers they could continue shopping as usual and retailers to conduct their businesses as usual. That advice turned out to be misguided. On July 8, government officials testing meat from a Tokyo slaughterhouse said they detected levels of radioactive cesium at nearly five times Japan's limit. The contaminated beef was traced to a farm about 10 miles north of the damaged plant—from an animal whose hide had been checked by inspectors.

The revelation has raised all kinds of questions about how much contaminated beef had already been consumed, kicking off a food scare that continues to grow as more tainted meat was discovered. *Wall Street Journal* examination showed that four months after the disaster, the government was still struggling to contain the contamination and to come up with an effective system for policing its food supply. Some foods, such as juices and honey, hit store shelves without any government screening. Many other foods were spot tested, but only minimally (*WSJ* 2011). The Japanese officials scrambled to figure out how dangerous it was and what they should do to protect the public. Low levels of radioactivity can do damage over the long term, and scientists say it is difficult to pinpoint the precise level at which such contamination becomes unacceptably risky.

Japan succeeded in catching some contaminated food before it reached store shelves, including milk and spinach from the Fukushima prefecture where the plant is located. But its spot-checking of beef from the troubled region turned out to be insufficient. Between the time of the disaster and the first discovery of tainted beef, the government figures indicate that only about 50 of the 10,000 (about 9.5) or so cattle shipped from Fukushima were tested.

In an effort to contain the problem, the government on July 19 asked Fukushima prefecture to halt all beef cattle sales. On July 28, it stopped cattle sales in the adjacent Miyagi, Iwate, and Tochigi prefectures as well.

The country is now facing the challenge of testing more beef that it may be equipped to handle. The animal to which the first tainted beef was traced had been fed rice straw exposed to radioactive fallout. The government determined that contaminated rice straw had been fed to many cattle that had since been slaughtered, meaning that tainted meat found its way to supermarkets, restaurants, and school cafeterias. The government was trying to track and test meat from nearly 3500 cattle it believed ate contaminated straw. Japanese Prime Minister Naoto Kan has admitted that the country's standards for ensuring beef safety are not stringent enough.

On July 5, 2001, Japan's cabinet unveiled a proposed supplementary budget to support recovery efforts from the March 11 disaster (*Science*, July 15, 2011).

On July 25, Japan's legislature approved a supplementary budget including $1.2 billion for health care and long-term studies of people exposed to radiation from the devastated Fukushima Daiichi nuclear power plant. Seiji Yasumura, a gerontologist at Fukushima Medical University was appointed director. He had already distributed questionnaires to identify those among the prefecture's 2 million residents whose radiation exposure could warrant closer examination.

Local newspapers reported that residents with significant exposure could be followed for 20 years. More importantly, each of the prefecture's 360,000 youngsters age 18 and under would have a thyroid examination because thyroid cancer among young people was the clearest health effect of the Chernobyl nuclear disaster of 1986. All 20,000 pregnant women of the prefecture would also be examined and their babies' health tracked. The plan also calls for medical check-ups for an estimated 200,000 people evacuated from the vicinity of the power plant as well as mental health support for those in need. This initiative is just the first phase of an effort expected to last at least 30 years.

Scientists in the world have long debated the health effects of chronic low radiation doses. They hope that Japan's research might yield insights into whether a threshold exists below which radiation exposure has not ill effect or is possibly beneficial (*Science* 2011).

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4 Toxic Action of Pollutants

4.1 INTRODUCTION

When present at a sufficiently high concentration, a pollutant can cause adverse effects on an organism. To elicit damage to an exposed organism, the pollutant must first enter the host and reach its target site. A complex pathway exists between the time of initial toxicant exposure and the manifestation of damage induced in the organism. This chapter discusses general ways in which environmental pollutants exert their actions on plants, animals, and humans.

4.2 EFFECTS ON PLANTS

4.2.1 SOURCE OF POLLUTANTS

For the most part, environmental pollution is an anthropogenic (human-made) problem. As mentioned previously, the most important source of atmospheric pollution in the United States is motor vehicles. Other major sources include industrial activities, power generation, space heating, and refuse burning. The composition of pollutants from different sources varies markedly, with industry emitting the most diverse range of pollutants. While carbon monoxide (CO) is the major component of pollution by motor vehicles, sulfur oxides (SO_X) are primary pollutants emitted by industry, power generation, and space heating. In some large cities, such as Los Angeles, ozone (O_3), peroxyacyl nitrate (PAN), and other photochemical oxidants produce major atmospheric pollution problems.

4.2.2 UPTAKE OF POLLUTANTS

Terrestrial plants may be exposed to environmental pollutants in two major ways. One is exposure of foliage to air pollutants; another is uptake of pollutants by roots growing in contaminated soils. Vegetation growing near industrial facilities, such as smelters, aluminum (Al) refineries, and coal-burning power plants, may absorb airborne pollutants through the leaves and become injured. The pollutants may be in gaseous form, such as sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and hydrofluoric acid (HF), or in particulate form, such as the oxides or salts of metals contained in fly ash (Figure 4.1).

The uptake of airborne pollutants by plants is important for examining their effects on vegetation. While the concentration of the atmospheric pollutant in question is an important factor, the actual amount that is taken up by the plant is more critical. The conductance through the stomata, which regulate the passage of ambient air into the cells, is especially crucial. The extent of uptake depends on the

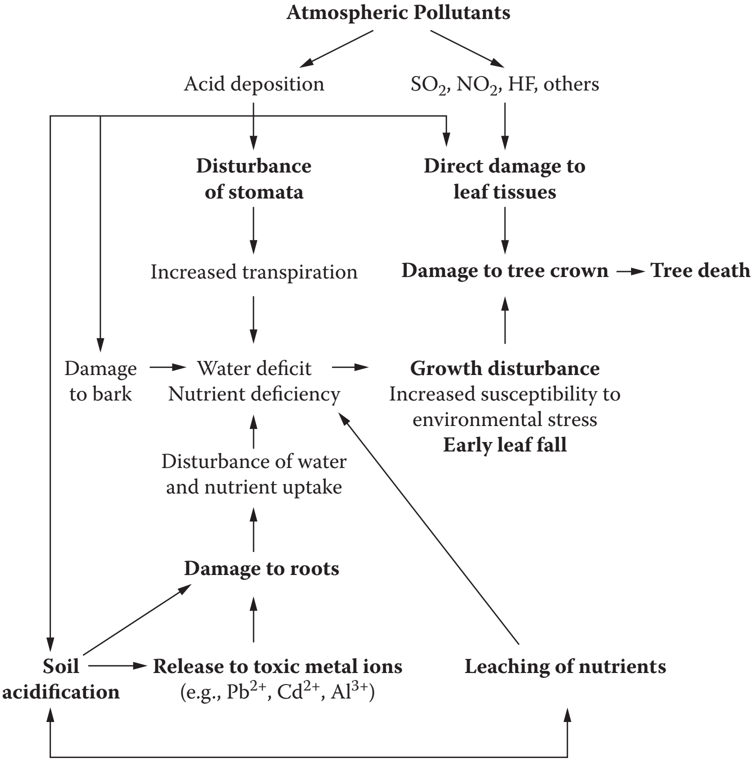

Atmospheric Pollutants
Acid deposition
SO2, NO2, HF, others
Disturbance of stomata
Direct damage to leaf tissues
Increased transpiration
Damage to tree crown
Tree death
Damage to bark
Water deficit
Nutrient deficiency
Growth disturbance
Increased susceptibility to environmental stress
Early leaf fall
Disturbance of water and nutrient uptake
Damage to roots
Soil acidification
Release to toxic metal ions (e.g., Pb2+, Cd2+, Al3+)
Leaching of nutrients

FIGURE 4.1 Mechanisms of tree damage by air pollutants.

chemical and physical properties of the pollutant along the gas-to-liquid diffusion pathway. The flow of a pollutant may be restricted by the physical structure of the leaf (Figure 4.2) or by scavenging reactions occurring in the leaf itself. Leaf orientation and morphology, including epidermal characteristics, and air movement across the leaf are important determinants affecting the initial flux of gases to the leaf surface. Stomatal resistance is an essential factor for determining pollutant uptake. The resistance is affected by stomatal size and number, the size of the stomatal aperture, and other anatomical characteristics (Black and Unsworth 1980). The stomatal opening is extremely important; little or no uptake may occur when the stomata are closed. It is regulated by light, humidity, temperature, internal CO_2 content, water and nutrient availability to the plant, and potassium ions (K^+) absorbed by the guard cells (Humble and Raschke 1971).

Exposure of roots to toxicants in contaminated soils is another important process by which toxicant uptake by plants occurs. For example, vegetation growing in soils of contaminated sites, such as waste sites and areas that have received application of contaminated sewage sludge, can absorb toxicants by the roots. In the contaminated sites, high levels of heavy metals such as lead (Pb) and cadmium (Cd) often occur. Metallic ions are more readily released, and thus more readily absorbed, when the soil is acidified by acid deposition (Figure 4.1).

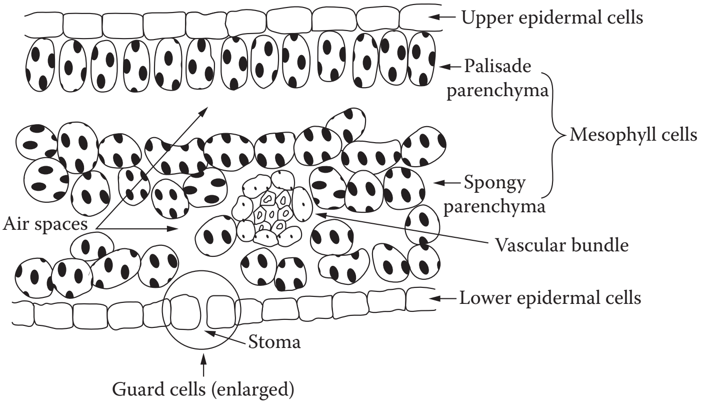


FIGURE 4.2 Cross section of a leaf showing the air spaces that serve as passages for pollutants.

4.2.3 TRANSPORT OF TOXICANT

Following uptake, a toxicant may mix with the surrounding medium of the plant and then be transported to various organs and tissues. Mixing involves the microscopic movement of molecules and is accompanied by compensation of concentration differences. Generally, transport of chemicals in plants occurs passively by diffusion and flux. Diffusion refers to movement across phase boundaries, from a high-concentration compartment to a low-concentration compartment. Flux, on the other hand, refers to the horizontal movement of the medium.

4.2.4 PLANT INJURY

Besides destroying and killing plants, air pollutants can induce adverse effects on plants in several ways. As mentioned previously, pollution injury is commonly divided into acute and chronic injury. In plants, an acute injury such as destruction of tissues occurs following absorption of sufficient amounts of toxic gas or other forms of toxicants. Tissue destruction is often manifested by collapsed leaf margins or other areas, exhibiting an initial water-soaked appearance. Subsequently, the leaf becomes dry and bleaches to an ivory color or becomes brown or brownish red. By contrast, a chronic injury may be caused by uptake of sublethal amounts of toxicants over a long period. Chronic injury is manifested by yellowing of leaves that may progress slowly through stages of bleaching until most of the chlorophyll and carotenoids are destroyed.

To induce leaf injury, an air pollutant has to pass through the stomata of the epidermal tissue, as the epidermis is the first target for the pollutant. In passing into the intercellular spaces, the pollutant may dissolve in the surface water of the leaf cells, causing changes in cellular pH. A pollutant may not remain in its original form as it passes into solution. Rather, it may be converted into a form that is more reactive and toxic than the original substance. The formation of reactive free radicals following the initial reaction in the cell is an example. The pollutant, either in its original form or in an altered form, may then react with specific cellular components, such as cytoplasmic membrane or membranes of the organelles, or with various substances, including enzymes, coenzymes or cofactors, and substrates. The pollutant may thus adversely affect cellular metabolism, leading to plant injury (Heath 1980).

An example of a gaseous air pollutant widely known to cause damage to plants is SO_2. Once absorbed into the leaf, SO_2 can induce injuries to the ultrastructure of various organelles such as chloroplasts and mitochondria, leading to disruption of photosynthesis or cellular energy metabolism. Similarly, histochemical studies of fluoride-induced injury have shown that the damage to leaves first occurs in the spongy mesophyll and lower epidermis, followed by distortion or disruption of chloroplast in the palisade cells (Miller et al. 1983).

As a pollutant moves from the substomatal regions to the cellular sites of perturbation, it may encounter various obstacles along the pathway. Scavenging reactions between endogenous substances and the pollutant may occur, and the result can affect pollutant toxicity. For example, ascorbate, which occurs widely in plant cells, may react with or neutralize a particular pollutant or a secondary substance produced as the pollutant is metabolized. Conversely, an oxidant such as O_3 may react with membrane material and induce peroxidation of the lipid components. This is followed by the formation of various forms of toxic substances, such as aldehydes, ketones, and free radicals (Grimes et al. 1983; Mehlman and Borek 1987). The free radicals, in turn, may attack cellular components, such as lipids, proteins, and nucleic acids, leading to tissue damage. Endogenous antioxidants, such as the ascorbic acid mentioned, may react with free radicals and alter their toxicity.

Cellular enzyme inhibition is often observed when leaves are exposed to atmospheric pollutants. The inhibition occurs even before the leaf injuries become apparent. For instance, fluoride (F), widely known as a metabolic inhibitor, can inhibit a large number of enzymes. Fluoride-induced enzyme inhibition is often attributable to interaction of F^- with certain metallic cofactors, such as Cu^{2+} or Mg^{2+} ions, in an enzyme system. Heavy metals, such as lead and cadmium, may also inhibit enzymes that contain a sulfhydryl (–SH) group at the active site. Alternatively, SO_2 may oxidize and break apart the sulfur bonds in critical enzymes of the membrane, thus disrupting cellular function.

Soil acidification increases release of toxic metal ions, such as Pb^{2+} and Cd^{2+} ions. These metal ions may directly damage roots by disrupting water and nutrient uptake, resulting in water deficit or nutrient deficiency. Soil acidification can also cause leaching of nutrients, leading to nutrient deficiency and growth disturbance (Figure 4.1). A plant becomes unhealthy as a result of one or more of the disturbances mentioned. Even before visible symptoms are discernible, an exposed plant may be weakened and its growth impaired. In time, visible symptoms such as chlorosis or necrosis may appear, leading to plant death.

4.3 MAMMALIAN ORGANISMS

4.3.1 EXPOSURE

An environmental pollutant may enter an animal or humans through a variety of routes. Figure 4.3 shows the general pathways that pollutants can follow in mammalian

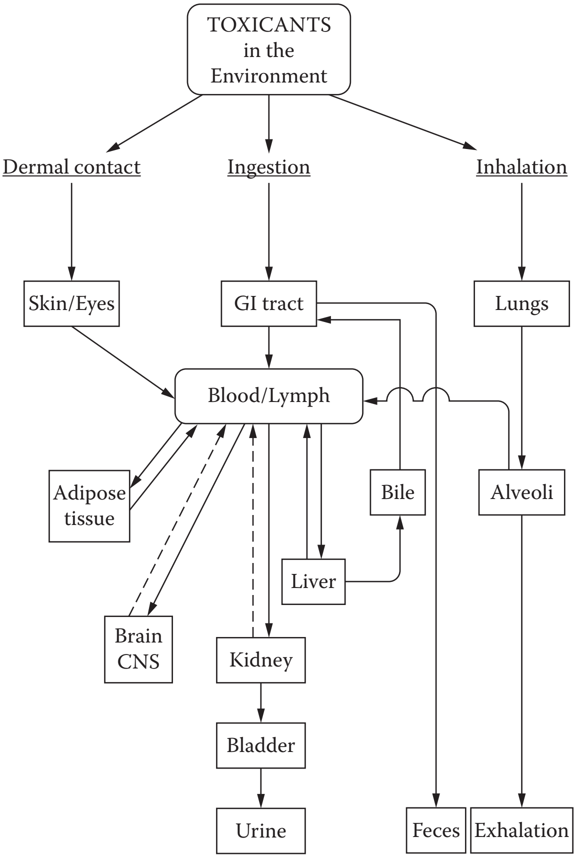

TOXICANTS in the Environment
Dermal contact
Ingestion
Inhalation
Skin/Eyes
GI tract
Lungs
Blood/Lymph
Adipose tissue
Bile
Alveoli
Liver
Brain CNS
Kidney
Bladder
Urine
Feces
Exhalation

FIGURE 4.3 Poisoning processes in humans/animals.

organisms. As mentioned previously, exposure of a host organism to a pollutant constitutes the initial step in the manifestation of toxicity. A mammalian organism may be exposed to a pollutant through inhalation, dermal contact, eye contact, or ingestion.

4.3.2 Uptake

The immediate and long-term effects of a pollutant are directly related to its mode of entry. The portals of entry for an atmospheric pollutant are the skin, eyes, lungs, and gastrointestinal tract. The hair follicles, sweat glands, and open wounds are the possible sites of entry where uptake from the skin may occur. Both gaseous and particulate forms of air pollutants can be taken up through the lungs. Uptake of toxicants by the gastrointestinal tract may occur when foods or beverages consumed are contaminated by air pollutants, such as lead, cadmium, or sprayed pesticides.

For a pollutant to be taken up into the body and finally carried to a cell, it must pass through several layers of biological membranes. These include not only the peripheral tissue membranes, but also the capillary and cell membranes. Therefore,

TABLE 4.1
Four Basic Types of Absorption Processes[a]

Process	Energy Requirement	Carrier	Concentration Gradient
Passive	No	No	High → Low
Facilitated	No	Yes	High → Low
Active	Yes	Yes	High → Low Low → High
Phagocytosis/pinocytosis	Yes	No	NA

Note: NA, not applicable.

[a] Phagocytosis is involved in invagination of solid particles, whereas pinocytosis refers to uptake of liquids.

the nature of the membranes and the chemical and physical properties (e.g., lipophilicity) of the toxicant in question are important factors affecting uptake. The mechanisms by which chemical agents pass through the membrane include

- filtration through spaces or pores in membranes
- passive diffusion through the spaces or pores or by dissolving in the lipid material of the membrane
- facilitated transport, by which a specialized protein molecule, called a *carrier,* carries a water-soluble substance across the membrane
- active transport, which requires both a carrier and energy

Of the four mechanisms shown, active transport is the only one in which a toxicant can move against a concentration gradient, that is, move from a low-concentration compartment to a high-concentration compartment (Table 4.1). This is the reason for the need for energy expenditure.

4.3.3 TRANSPORT

Immediately after absorption, a toxicant may be bound to a blood protein (such as lipoprotein), forming a complex, or it may exist in a free form. Rapid transport throughout the body follows. Transport of a toxicant may occur through the bloodstream or lymphatic system. The toxicant may then be distributed to various body tissues, including those of storage depots and sites of metabolism.

4.3.4 STORAGE

A toxicant may be stored in the liver, lungs, kidneys, bone, or adipose tissue. These storage depots may or may not be the sites of toxic action, however. A toxic agent may be stored in a depot temporarily and then released and translocated again. Similarly, a toxicant or its metabolite may be transported to a storage site and remain there for a long period of time or permanently. Excretion of the toxicant following temporary storage may also occur.

4.3.5 METABOLISM

The metabolism of toxicants may occur at the portals of entry or in such organs as the skin, lungs, liver, kidney, and gastrointestinal tract. The liver plays a central role in the metabolism of environmental toxicants (xenobiotics). The metabolism of xenobiotics is often referred to as *biotransformation*. The liver contains a rich supply of nonspecific enzymes, enabling it to metabolize a variety of organic compounds.

Biotransformation reactions are classified into two phases: phase I and phase II (Yu 2004). Phase I reactions are further divided into three main categories: oxidation, reduction, and hydrolysis. These reactions are characterized by the introduction of a reactive polar group into the xenobiotic, forming a primary metabolite. In contrast, phase II reactions involve conjugation reactions in which the primary metabolite combines with an endogenous substance, such as certain amino acids or glutathione (GSH), to form a complex secondary metabolite. The resultant secondary metabolite is more water soluble and therefore more readily excreted than the original toxicant.

While many xenobiotics are detoxified as a result of these reactions, others may be converted to more active and more toxic compounds. Biotransformation is discussed in more detail in Chapter 6.

4.3.6 EXCRETION

The final step in the pathway of a toxicant is excretion from the body (Figure 4.3). A toxicant may be excreted through the lungs, kidneys, or intestinal tract. Toxicant excretion may occur in the original form of the toxicant or as its metabolite(s), depending on its chemical property. Excretion is the permanent means by which toxic substances are removed from the body.

4.4 MECHANISM OF TOXIC ACTION

The toxic action of pollutants involves compounds with intrinsic toxicity or activated metabolites. These interact with cellular components at their site of action to induce toxic effects. These effects may occur anywhere in the body. The consequences of such action may be shown by changes in physiological and biochemical processes within the exposed organism. These changes may be manifested in different ways, including impaired central nervous system (CNS) function and oxidative metabolism, injury to the reproductive system, or altered DNA leading to carcinogenesis.

The duration of toxic action depends on the characteristics of the toxicant and the physiological or biochemical functioning of the host organism. Generally, the toxic action of a xenobiotic may be terminated by storage, biotransformation, or excretion.

The mechanisms involved in xenobiotic-induced toxicity are complex, and much remains to be elucidated. The ways in which xenobiotics can induce adverse effects in living organisms include

- disruption or destruction of cellular structure
- direct chemical combination with a cell constituent
- inhibition of enzymes
- initiation of a secondary action
- free-radical-mediated reactions
- disruption of reproductive function

These mechanisms are examined in the following sections.

4.4.1 Disruption or Destruction of Cellular Structure

A toxicant may induce an injurious effect on plant or animal tissues by disrupting or destroying the cellular structure. As mentioned, atmospheric pollutants, such as SO_2, NO_2, and O_3, are phytotoxic—they can cause plant injuries. Sensitive plants exposed to any of these pollutants at sufficiently high concentrations may exhibit structural damage when their tissue cells are destroyed. Studies show that low concentrations of SO_2 can injure epidermal and guard cells, leading to enhanced stomatal conductance and greater entry of the pollutant into leaves (Black and Unsworth 1980). Similarly, after entry into the substomatal cavity of the plant leaf, O_3, or the free radicals produced from it, may react with protein or lipid membrane components, disrupting the cellular structure of the leaf (Heath, 1980; Grimes et al. 1983). In animals and humans, inhalation of sufficient quantities of NO_2 and H_2SO_4 mists can damage surface layers of the respiratory system. Similarly, high levels of O_3 can induce peroxidation of the polyunsaturated fatty acids in the membrane lipids, resulting in disruption of membrane structure (Mehlman and Borek 1987).

4.4.2 Direct Chemical Combination with a Cell Constituent

A pollutant may combine with a cell constituent, forming a complex and disrupting cellular metabolism. For example, CO is widely known for its ability to bind to hemoglobin (Hb). After its inhalation and diffusion into the blood, CO readily reacts with Hb to form carboxyhemoglobin (COHb):

$$\mathrm{CO} + \mathrm{Hb} \rightarrow \mathrm{COHb} \tag{4.1}$$

The presence of a large amount of COHb in the blood disrupts the vital system for exchange of CO_2 and O_2 between the blood and the lungs and other body tissues. Interference with the functioning of hemoglobin by COHb accumulation is detrimental to health and can lead to death.

A number of toxicants or their metabolites are capable of binding to DNA to form DNA adducts. Formation of such adducts results in structural changes in DNA, leading to carcinogenesis. For instance, benzo[α]pyrene, one of the many polycyclic aromatic hydrocarbons (PAHs), may be converted to its epoxide form in the body. The resultant epoxide can in turn react with guanine on a DNA molecule to form a guanine adduct. Another example is found with alkylating agents. These chemicals are metabolized to reactive alkyl radicals, which can also induce adduct formation. These are discussed in more detail in Chapter 16.

Certain metallic cations can interact with the anionic phosphate groups of polynucleotides. They can also bind to polynucleotides at various specific molecular sites, particularly purines and thymine. Such metallic cations can, therefore, inhibit DNA replication and RNA synthesis and cause nucleotide mispairing in polynucleotides. An anatomical feature of chronic lead intoxication in humans and in various animals is the presence of characteristic intranuclear inclusions in proximal tubular epithelial cells in the kidneys. These inclusions appear to be formed from lead and soluble proteins (Choie and Richter 1972). Through tying up cellular proteins, lead can depress or destroy their function.

4.4.3 Effect on Enzymes

The most distinctive feature of reactions that occur in living cells is the participation of enzymes as biological catalysts. Almost all enzymes are proteins with a globular structure, and many of them carry out their catalytic function by relying solely on their structure. Many others require nonprotein components, called cofactors. Cofactors may be metal ions; alternatively, they may be organic molecules, called coenzymes. Metal ions capable of acting as cofactors include K^+, Na^+, Cu^{2+}, Fe^{2+} or Fe^{3+}, Mg^{2+}, Mn^{2+}, Ca^{2+}, and Zn^{2+} ions (Table 4.2). Examples of coenzymes that serve as transient carriers of specific atoms or functional groups are presented in Table 4.3. Many coenzymes are vitamins or contain vitamins as part of their structure. Usually, a coenzyme is firmly bound to its enzyme protein, and it is difficult to separate the two. Such tightly bound coenzymes are referred to as *prosthetic groups* of the enzyme. The catalytically active complex of protein and prosthetic group is called a *holoenzyme,* while the protein without the prosthetic group is called the *apoenzyme,* which is catalytically inactive.

TABLE 4.2
Examples of Enzymes that Require Metallic Ions

Metallic Ion	Enzyme
Ca^{2+}	Lipase, α-amylase
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Catalase; cytoytochrome oxidase/peroxidase
K^+	Pyruvate kinase
Mg^{2+}	Hexokinase, enolase, pyruvate kinase
Se^{2+}	Glutathione peroxidase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase; DNA polymerase

TABLE 4.3
Coenzymes Serving as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Entity Transferred
Coenzyme A	Acyl group
Flavin adenine dinucleotide	Hydrogen atoms
Nicotinamide adenine dinucleotide	Hydride ion (H^-)
Thiamin pyrophosphate	Aldehyde
Biotin	CO_2

$$\underset{(\textit{Apoenzyme})}{\text{Enzyme + Prosthetic group}} \rightarrow \underset{(\textit{Holoenzyme})}{\text{Protein-prosthetic group}} \tag{4.2}$$

Coenzymes are especially important in animal and human nutrition because, as mentioned, most are vitamins or are substances produced from vitamins. For example, niacin, after being absorbed into the body, is converted to nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH), important coenzymes in cellular metabolism.

There are several ways in which toxicants can inhibit enzymes, leading to disruption of metabolic pathways. Some examples are given next.

4.4.3.1 Enzyme Inhibition by Inactivation of Cofactor

As mentioned, some cofactors in an enzyme system are metallic ions, which provide electrophilic centers in the active site, facilitating catalytic reactions. For instance, fluoride has been shown to inhibit α-amylase, an enzyme responsible for the breakdown of starch into maltose and eventually glucose. The released glucose is then used as an energy source. α-Amylase is known to require Ca^{2+} for its stability as well as catalytic action (Schwimmer and Balls 1949; Beers and Duke 1990). The activity of α-amylase is inhibited in the presence of F^- ions (Sarkar et al. 1982; Yu et al. 1988). The inhibition appears to involve a mechanism by which the Ca^{2+} cofactor is removed by F^- ions. Evidence supporting this observation was obtained when a crude enzyme extract from mung bean seedlings exposed to 5 m*M* NaF for 3 days was tested for activity. When the enzyme extract was incubated with $CaCl_2$ for 30, 60, and 90 minutes, α-amylase activity was much higher than the activity shown by the assay mixture without the addition of $CaCl_2$ (Figure 4.4) (Yu et al. 1988).

Fluoride is also known to inhibit enolase, an enzyme involved in glycolysis. Enolase requires Mg^{2+} as a cofactor (see Chapter 10, Reaction 10.7). The fluoride-induced inhibition of the enzyme is more marked in the presence of phosphate. It is therefore assumed that the mechanism involved in the inhibition is through inactivation of the cofactor magnesium by F^-, resulting from the formation of magnesium-fluorophosphate.

4.4.3.2 Enzyme Inhibition by Competition with the Cofactor

Many enzymes carry out their catalytic function depending solely on their protein structure. Many others require nonprotein cofactors for their functioning. Cofactors

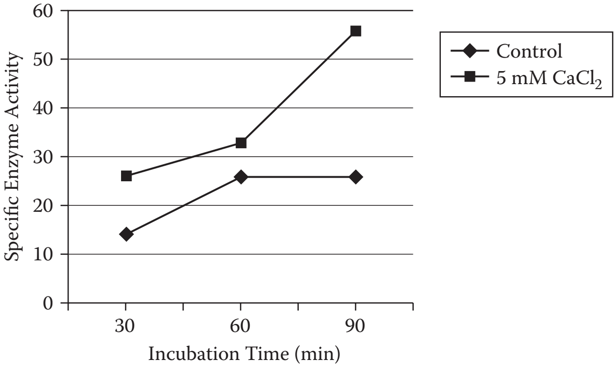


FIGURE 4.4 Effect of Ca on α-amylase activity in mung bean seedlings exposed to NaF. Enzyme assay mixture contained Tris-buffer (pH 7.0), 0.2% starch solution, and water (control) or 5 m*M* $CaCl_2$, and the mixture was incubated for 30, 60, and 90 minutes, respectively. Glucose produced at each incubation period was determined for specific enzyme activity. (*Source*: Data from Yu, M.H., M. Shumway, and A. Brockbank. Effects of NaF on amylase in mung bean seedlings. *J. Fluorine Chem.* 41, 95. 1988.)

may be metal ions or organic molecules referred to as coenzymes. Table 4.2 shows several metal ions and some enzymes that require them, while examples of several coenzymes and representative enzymes using the coenzymes are presented in Table 4.3. As shown in the Table 4.2, several enzymes require Zn^{2+} ions as a cofactor. Cadmium (Cd^{2+}), which is chemically similar to Zn^{2+}, can inhibit these enzymes by competing with the Zn^{2+} cofactor. Beryllium (Be) is known to inhibit certain enzymes that require Mg^{2+} for a similar reason.

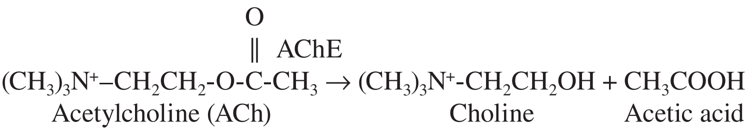
4.4.3.3 Enzyme Inhibition by Binding to the Active Site

A toxicant may bind to the active site of an enzyme. For instance, a thiol or sulfhydryl (–SH) group on a protein enzyme often is the active site for the catalytic action of the enzyme. A heavy metal, such as lead, cadmium, or mercury, after absorption into the body may attach itself to the –SH group, forming a covalent bond with the sulfur atom (Reaction 4.3). With the active site being blocked, the activity of the enzyme will be depressed or lost.

$$2\ Enz\text{–SH} + Pb^{2+} \rightarrow Enz\text{-}S\text{-Pb-}S\text{-}Enz + 2H^+ \tag{4.3}$$

For example, alanine aminotransferase (the enzyme that catalyzes the transamination of alanine) and δ-aminolevulinic acid dehydratase (ALA-D, a key enzyme in the heme synthetic pathway) both have the –SH group as the active site. Lead strongly inhibits both of these enzymes through the same mechanism.

Another example is the widely known inhibition of acetylcholinesterase (AChE) by chemicals such as organophosphate. AChE is the enzyme responsible for the breakdown of acetylcholine (ACh), the neurotransmitter in insect and vertebrate nervous systems (Reaction 4.4). When AChE is inhibited, ACh will accumulate and keep firing at the nerve endings. As a result, the nerve functioning is interrupted, which may lead to death of the affected organism.



$$\underset{\text{Acetylcholine (ACh)}}{(CH_3)_3N^+\text{–}CH_2CH_2\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH_3} \xrightarrow{AChE} \underset{\text{Choline}}{(CH_3)_3N^+\text{-}CH_2CH_2OH} + \underset{\text{Acetic acid}}{CH_3COOH} \qquad (4.4)$$

Evidence suggests that the vertebrate AChE contains two binding sites; one of them is serine (an amino acid) with the $–CH_2OH$ residue as the active site. Chemicals such as organophosphate pesticides, which can inactivate AChE, are known to attach to the functional group $–CH_2OH$ in serine on the enzyme molecule by forming a covalent bond (see Section 13.2.3.3).

4.4.3.4 Inhibition of Enzyme Activity by Toxic Metabolite

In this case, enzyme inhibition is not caused by the toxicant itself; rather, it is caused by the metabolite. For example, sodium fluoroacetate, known as Rat Poison 1080, is extremely toxic to animals. However, the toxicity is not due to sodium fluoroacetate itself but is rather due to a product formed in the metabolism, that is, fluorocitrate. This product is formed through a reaction commonly known as *lethal synthesis* (Figure 4.5). The resultant fluorocitrate is toxic because it is a potent inhibitor of aconitase, the enzyme that catalyzes the conversion of citrate into *cis*-aconitate and then into isocitrate (in the TCA [tricarboxylic acid] cycle). Inhibition of aconitase results in citrate accumulation. The outcome of this inhibition is an impaired TCA cycle function, leading to disruption of energy metabolism.

4.4.4 SECONDARY ACTION AS A RESULT OF THE PRESENCE OF A POLLUTANT

The presence of a pollutant in a living system may cause the release of certain substances that are injurious to cells. Several examples follow.

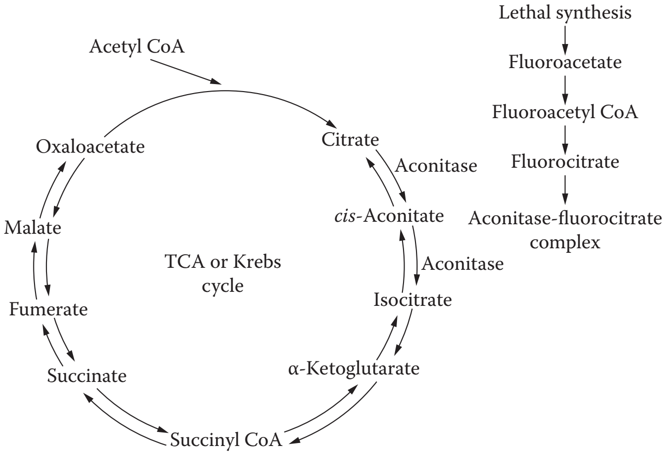

Lethal synthesis
Fluoroacetate
Fluoroacetyl CoA
Fluorocitrate
Aconitase-fluorocitrate complex
Acetyl CoA
Oxaloacetate
Citrate
Aconitase
cis-Aconitate
Aconitase
Malate
TCA or Krebs cycle
Isocitrate
Fumerate
Succinate
α-Ketoglutarate
Succinyl CoA

FIGURE 4.5 Synthesis of fluorocitrate from fluoroacetate through lethal synthesis. Inhibition of aconitase shuts down the TCA cycle.

4.4.4.1 Allergic Response to Pollen

In many individuals, allergic response occurs after inhalation of pollen, leading to common symptoms of hay fever. The symptoms are due to the release of histamine, a substance formed from the amino acid histidine through decarboxylation. Histamine is made and stored in the mast cell and in many other cells of the body. Release of histamine occurs in anaphylaxis or as a consequence of allergy; it is also triggered by certain drugs and chemicals. Histamine is a powerful vasodilator, capable of causing dilation and increase in blood vessel permeability. Histamine also stimulates pepsin secretion and can reduce the blood pressure and, if severe enough, induce shock. When present in excessive levels, histamine can cause vascular collapse. Antihistamines, such as diphenylhydramine and antergan, are compounds whose structures are similar to that of histamine and can prevent physiological changes induced by histamine.

4.4.4.2 Carbon Tetrachloride

The way in which carbon tetrachloride ($CC1_4$) affects humans is another example of secondary action as a result of presence of a pollutant. Once taken up into the body, $CC1_4$ is known to cause a massive discharge of epinephrine from sympathetic nerves, eventually inducing liver damage. Epinephrine is a potent hormone, involved in many critical biological reactions in animals and humans, including

- stimulation of glycogenolysis (breakdown of glycogen into glucose) in the liver and muscle. In the liver, the resultant glucose enters blood circulation; in the muscle, the resultant glucose does not enter blood circulation but instead is converted to lactic acid before being transferred back to the liver.
- lipolysis (breakdown of fats). This involves the breakdown of triacylglycerol into fatty acids and glycerol.
- glucagon secretion.
- inhibition of glucose uptake by muscle.
- insulin secretion.

Epinephrine also increases blood pressure. Like other hormones, epinephrine is rapidly broken down when its function is finished. The breakdown of epinephrine occurs mainly in the liver. It has been shown that, in the liver, $CC1_4$ is broken down into reactive free radicals, that is, $\cdot CC1_3$ and $Cl\cdot$ (Reaction 4.5). It is suggested that the free radicals in turn can damage liver by reacting with its cellular components.

$$\begin{array}{c}\text{Cytochrome P450}\\ CC1_4 \rightarrow \cdot\, CC1_3 + Cl\cdot\end{array} \tag{4.5}$$

4.4.4.3 Chelation

Chelation is a process by which atoms of a metal in solution are sequestered by ring-shaped molecules. The ring of atoms, usually with oxygen, nitrogen, or sulfur as an electron donor, has the metal as an electron acceptor. The metal is more firmly gripped within this ring than if it were attached to separate molecules. The formation of strain-free stable chelate rings requires at least two atoms that can attach to a metal ion. The iron (Fe) in a hemoglobin molecule and the magnesium in a chlorophyll molecule are two such examples. Through chelation, some biologically active compounds are absorbed and retained in the body, whereas others may be removed from it.

Some researchers suggest that the toxicity of certain chemicals may be attributed to chelation. For instance, when rabbits were exposed to carbon disulfide (CS_2) at 250 ppm, a rapid outpouring of tissue zinc (Zn) in urine occurred. The loss of body zinc is primarily due to a chemical reaction of CS_2 with free amino groups of tissue protein, forming thiocarbamate and thiazolidone, which might form soluble chelate with zinc (Talegawkar et al. 2009).

Some scientists suggested that metal chelation may be one of the mechanisms involved in carcinogenesis. Many carcinogens have, or can be metabolized to, chemical species capable of metal binding. This in turn may aid the entrance of metals into cells. Once inside the cells, interaction between normal metals and abnormal metals may occur, resulting in alteration of cellular metabolism.

4.4.4.4 Metal Shift

Metal shift refers to movement of metals from one organ to another due to the presence of a toxicant. The phenomenon may account for some of the responses observed in animals that are exposed to certain toxicants and is among the earliest biological indicators of toxic response. For example, rats exposed to fluoride showed an increase in serum zinc content, whereas the levels of selenium (Se) and aluminum in the rats' whiskers were decreased (Yoshida et al., 1991). A similar change was observed with rats exposed to O_3. When exposed to O_3 for 4 hours, the rats showed increased levels of copper (Cu), molybdenum (Mb), and zinc in their lungs, while the levels of these metals in the liver were decreased.

4.4.5 FREE-RADICAL-MEDIATED REACTIONS

A free radical is any molecule with an odd number of electrons and can occur as both organic and inorganic molecules. Free radicals are highly reactive and therefore highly unstable and short-lived. For instance, the half-life of lipid peroxyl radical (ROO·) is 7 seconds, and that of hydroxyl radical (HO·) is 10^{-9} seconds.

Free radicals are derived from both natural and anthropogenic sources. They are produced naturally in vivo as by-products from normal metabolism. Some of the examples include superoxide free radical ($O_2\cdot^-$) and H_2O_2. Anthropogenic sources of free radicals are found in such situations as when an organism is exposed to ionizing radiation, certain drugs, or various xenobiotics. The free radicals thus produced can cause chain reactions and damage critical cellular constituents, including proteins, lipids, and DNA. In proteins, the consequence of free-radical attacks is manifested by peptide-chain scission and denaturation. With DNA, strand scission or base modification may occur, potentially leading to cell mutation and death. Researchers generally agree that many human diseases, including heart disease and certain types of cancer, are attributable, at least partly, to free-radical-mediated reactions.

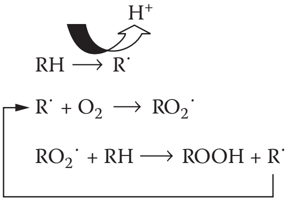


FIGURE 4.6 Lipid peroxidation and production of lipid free radicals RH, polyunsaturated fatty acid; R·, lipid (fatty acid) free radical; ROO·, lipid peroxide free radical; ROOH, lipid/organic hydroperoxide.

As free radicals react with unsaturated fatty acids and cholesterol, such as those in cellular membranes, they can induce lipid peroxidation. This process, in turn, can become autocatalytic after initiation, leading to the production of lipid peroxide, lipid alcohol, aldehydes, and other chemical species (Freeman and Crapo 1982). Interaction with other cellular constituents can also occur, thus injuring cells. Obviously, by inducing these reactions, free radicals can damage cell plasma membranes and those of organelles.

Certain atmospheric pollutants, such as O_3, PAN, and NO_2, can act as free radicals themselves. Extensive studies have been conducted on the nature of O_3-dependent peroxidation of lipid material in both plants and animals. Lipid peroxidation can also occur as a result of free-radical-dependent reactions initiated by other environmental agents. Figure 4.6 shows the mechanism involved in lipid peroxidation. It also shows the initiation of a chain reaction that can occur following the formation of new species of free radical. As a result of peroxidation and subsequent reactions, the nature of lipid material is altered, and cellular functions are disrupted.

Oxidative stress is an imbalance between the production of reactive oxygen radicals and the ability of the organism's natural protective mechanisms to cope with these radicals and to prevent adverse effects. Cells in the body are exposed to reactive oxygen species under normal circumstances via the leakage of electrons from the electron transport chain, phagocytic cells, and endogenous enzyme systems (Freeman and Crapo 1982).

Studies showed that free radicals such as the hydroxyl radical (OH·) can cause peroxidation or crosslinking of membrane lipids and intracellular compounds, thus leading to cell aging and death. Although this is part of the normal aging process of cells, the presence of increased oxidative stress is thought to lead to premature cell aging. For example, the potentially harmful reactivity and oxidative potential of iron are carefully modulated within living organisms by the binding of iron to carrier proteins or by the presence of other molecules with antioxidant properties. When not properly controlled, redox reactions can cause major damage to cellular components, such as fatty acids, proteins, and nucleic acids. Iron catalyzes the Fenton reaction, one of the best-known processes for converting superoxide and hydrogen peroxide to very reactive free radicals (Reactions 4.6 and 4.7).

$$O_2 + Fe^{+3} \rightarrow O_2^- + Fe^{+2} \tag{4.6}$$

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH\cdot \tag{4.7}$$

4.4.6 ENDOCRINE DISRUPTION

Estrogen, a steroid hormone, is produced in both males and females. It is produced in much larger quantities in females and therefore is considered a female hormone. In both humans and animals, a specific ratio of estrogen to androgens (male hormones) is necessary for sexual differentiation in the developing fetus. If the ratio is perturbed, the offspring may be born with two sets of partially developed sexual organs, called *intersex*, or with a single set that is incompletely or improperly developed.

Estrogenicity is mediated by binding to specific intracellular proteins known as *receptors*. This binding causes a conformational change in the receptor, enabling the estrogen-estrogen receptor complex to bind to specific sites on DNA. Once bound to DNA, the complex alters the expression of estrogen-responsiveness genes. Steroidal estrogens exert their effects through this change in gene expression (Figure 4.7). An exogenous chemical agent can alter the receptor-mediated process by a number of mechanisms. For example, the chemical agent may change the level of endogenous estrogen at a particular site by altering its synthesis, metabolism, distribution, or clearance. Alternatively, the chemical may modify tissue responsiveness to estrogen by changing receptor levels or by acting through a secondary pathway to influence the receptor function. Finally, a chemical may attach itself to the estrogen receptor in cells and mimic or block estrogenicity (Hileman 1994). Endocrine disrupters are therefore defined as exogenous chemical agents that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones (Environmental Protection Agency [EPA] 1997).

A particular group of chemicals, called estrogen mimics, can imitate the action of estrogen. The estrogen mimics are a diverse range of chemicals with no obvious

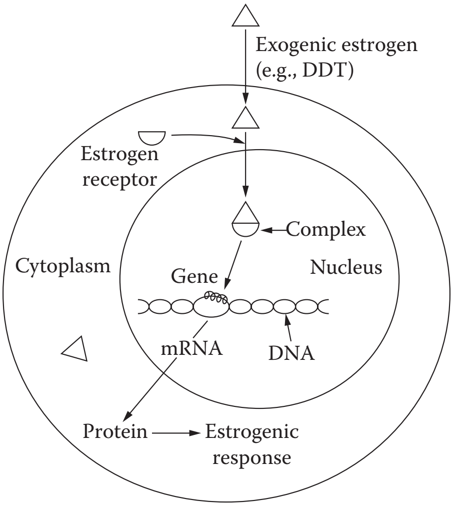


FIGURE 4.7 Impact of exogenic estrogen.

structural similarity. Nevertheless, major characteristics of these chemicals have been elucidated. These chemicals are highly persistent, highly fat-soluble, and have a high potential to accumulate in fat tissue of animals and humans. Some examples of estrogen mimics include DDT, DDE, dieldrin, Kepone, methoxychlor, and polychlorinated biphenyls (Shultz et al. 1998).

For example, DDT has been shown to cause reproductive failure in western gulls in California (Fry and Toone 1981). The poor breeding success was characterized by a reduced number of adult males, a highly skewed sex ratio (e.g., female-to-male ratios of 3.85 on Santa Barbara Island), and female-female pairing of some of the excess females. Researchers suggested that the causes for the observed poor breeding success might include DDT contamination, causing the thinning of eggshells and abnormal development of the reproductive system in embryos, leading to breeding failure in the adult birds.

Research conducted since 1990 indicated that certain persistent toxicants may be producing adverse effects in wildlife, including birds and mammals, and in humans by disrupting the endocrine system. Some of the effects include reproductive and developmental abnormalities, increases in certain hormone-related cancers, and decreases in wildlife populations.

Similarly, a number of other reports have indicated that sperm counts in men worldwide have decreased about 50% since 1940. Over the same period, the incidence of prostate cancer in some countries has doubled, while that of testicular cancer has tripled. There are also indications that birth defects in the male reproductive tract have increased over the past several decades. Furthermore, since 1940, the incidence of female breast cancer has risen in the United States and Western Europe. Studies also showed that endometriosis (the growth outside the uterus of cells that normally line the uterus), formerly a rare condition, now afflicts 5 million American women. Women who are afflicted by the disease in their reproductive years frequently suffer infertility.

In the animal world, a study of alligators on Lake Apopka in Florida found that the young were often unable to hatch, and that males that did hatch had abnormally small penises. An active program of research followed the observation, and a large number of reports related to the subject have been published. Many scientists agree that at least part of the reason for the observed conditions may be the introduction of xenobiotics into the environment since 1940 that block or mimic the action of estrogen.

Such chemicals may act on adult humans or animals, causing cancer or endometriosis. The consequences may be even more widespread and devastating when estrogen mimics accumulate in the mother. The chemicals may then be transferred to the egg or fetus, disrupting the hormone balance of the developing offspring and causing reproductive abnormalities or changes that set the stage for cancer in adulthood. (Further discussion of endocrine disruption is presented in Chapter 15.)

REVIEW QUESTIONS

1. Which is more injurious to plants/animals exposed to pollutants, continuous or intermittent exposure?
2. Explain how SO_2 may damage leaf tissues.
3. Explain the relationship between acid rain and plant injury.

4. Why is acidified soil more harmful to plants than nonacidified soil?
5. Explain the way in which lead may inhibit an enzyme.
6. Explain the way in which fluoride may inhibit an enzyme.
7. What is meant by facilitated transport?
8. To what does active transport refer? What are the characteristics involved in this process?
9. List the three main reactions involved in the phase I reaction.
10. Explain the main feature involved in the phase II reaction.
11. List four endogenous substances that may be involved in conjugation reactions.
12. Explain how a toxicant may disrupt DNA function.
13. Explain how cell membranes may be disrupted by lead or cadmium.
14. List several metallic ions that can act as a cofactor in an enzyme system.
15. What is a free radical? How is it produced?
16. Explain the way in which cellular macromolecules may be affected by free radicals.
17. What is meant by an estrogen mimic?
18. Name five chemicals that can act as estrogen mimics.
19. What are the major characteristics of estrogen mimics?

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5 Factors Affecting Xenobiotic Action

5.1 INTRODUCTION

Many factors can affect the toxicity of xenobiotics. This chapter examines some of the factors, including physicochemical properties of toxicants, dose or concentration, mode and duration of exposure, environmental factors, interaction, and biological and nutritional factors.

5.2 PHYSIOLOGICAL PROPERTIES

Physical and chemical characteristics—such as whether a pollutant is solid, liquid, or gas; whether it is soluble in water or in lipid, organic or inorganic material, ionized or nonionized, and so on—can affect the ultimate toxicity of pollutants. For instance, a nonionized substance may be more toxic than an ionized or charged counterpart because the nonionized species can pass through the membrane more easily than the ionized species and thus be more readily absorbed and elicit its toxic action.

5.3 DOSE OR CONCENTRATION

Dose or concentration of any pollutant to which an organism is exposed is often the most important factor affecting its toxicity. Once a pollutant gains entry into a living organism and reaches its target site, it may exhibit an injurious action. For this reason, any factors capable of modifying internal concentrations of the pollutant can alter its toxicity. The effect of the pollutant is therefore a function of its concentration at the locus of its action. A pollutant may either depress or stimulate normal metabolic function. In general, minute amounts of a pollutant may stimulate metabolic function, whereas large doses may impede or destroy its activity.

For example, epidemiological studies showed that in the area of Kuitan, a city situated in the western part of China, many residents suffer from arsenism (a disease caused by arsenic [As]) after consuming well water containing high levels of the mineral. Residents who had consumed well water containing 0.12 mg arsenic/L for 10 years manifested arsenism with a prevalence rate of 1.4% of the city population. However, in residents who had consumed water containing 0.6 mg arsenic/L for only 6 months, the prevalence rate increased to 47%, and the patients showed more severe symptoms (Wang et al. 1997).

Plants exposed to different kinds of pollutants often show depressed growth or lowered enzyme activity. For example, mung bean seedlings exposed to varying concentrations of sodium fluoride (NaF) for 3 days showed significant decreases

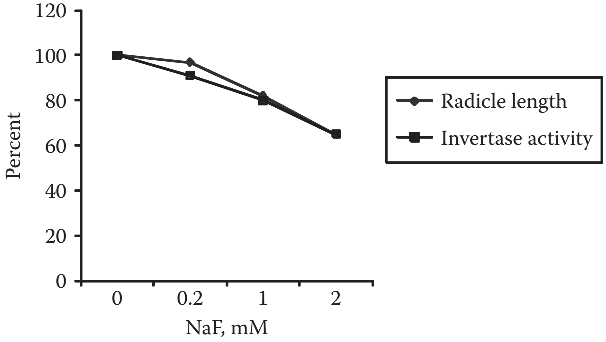


FIGURE 5.1 Effect of NaF on radicle growth and invertase activity in mung bean seedlings.

in root elongation and the activity of invertase, which is a key enzyme responsible for the breakdown of sucrose into glucose and fructose. Invertase activity in seedlings exposed to 0.2, 1.0, and 2.0 m*M* NaF was decreased by 9%, 22%, and 41%, respectively, compared to the control treated with water. These results coincided with impaired seedling growth (Figure 5.1) (Ouchi et al. 1999).

While it is true that exposure of organisms to sufficiently high levels of pollutants generally results in impaired growth or depressed enzyme activity, in a dose- or concentration-dependent manner, this is not always the case. Occasionally, under certain experimental conditions, increases in a certain endpoint (a measurable response of an organism to a stressor that is related to the evaluated characteristics chosen for assessing toxicity) may be observed in exposure studies in which very low concentrations of toxicants are used. Increases in respiration and growth rate are examples. Occasionally, increases in enzyme activity may also be observed. For example, mung bean seedlings exposed to 0.1 m*M* NaF showed an increase in superoxide dismutase (SOD) activity, whereas exposure to 0.5 m*M* and above showed decreases in the enzyme activity (Wilde and Yu 1998) (Figure 5.2). Some researchers have interpreted the observed increase as a result of the attempt by the organism to restore homeostasis by counteracting the stresses induced by toxicants. Such an attempt by the organism is almost always accompanied by an additional energy expenditure and therefore increased metabolism of the organism.

5.4 DURATION AND MODE OF EXPOSURE

The responses of an organism to stresses caused by toxicants are greatly affected by the duration of exposure. Ordinarily, a long-term exposure leads to a more severe injury than a short-term exposure. However, the dose or concentration of a toxicant is also important in determining the severity of the injury.

The mode of exposure of plants or animals to toxicants (i.e., continuous or intermittent) and the activity level of an exposed animal are also important in affecting pollutant toxicity. Normally, a continuous exposure is more injurious than an intermittent one, with other factors remaining the same. For instance, rats exposed

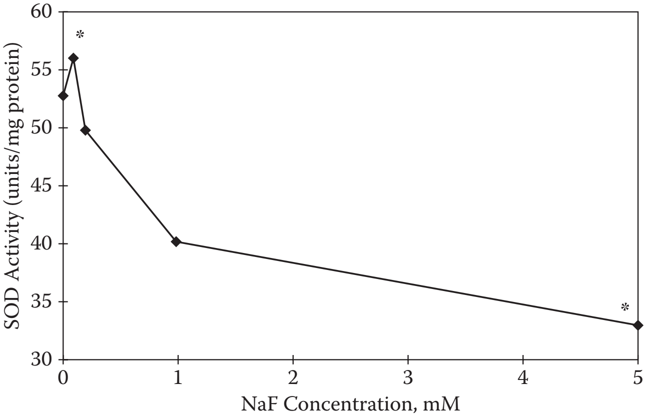


FIGURE 5.2 Effect of in vivo NaF exposure on SOD activity in mung bean seedlings.

to ozone (O_3) continuously for a sufficient period may develop pulmonary edema. However, when the animals are exposed to the same dose of O_3 administered intermittently, no pulmonary edema may occur. A similar phenomenon can also be observed with plants exposed to various kinds of air pollutants. One reason for this is that living organisms often can, to some extent, repair injuries caused by environmental chemicals. The magnitude of the health effects of O_3 on animals is also highly dependent on the activity level of the subject. Exercise increases the total volume of inhaled air, so it will also increase the total dose of O_3 to the lung. The duration of the exercise is more important than the dose of the exposure (Folinsbee et al. 1988).

5.5 ENVIRONMENTAL FACTORS

Environmental factors, such as temperature, pH, humidity, and others, may affect pollutant toxicity in different ways. Some of these factors are examined next.

5.5.1 Temperature

Many reports have shown the effects of changes of temperature on living organisms (Krenkel and Parker 1969). Changes in ambient temperature affect the metabolism of xenobiotics in animals. For example, the rate at which chemical reactions occur increases with increase in temperature. With fish, an increase in temperature leads to faster assimilation of waste and therefore faster depletion of oxygen. Fish and other aquatic life can live only within certain temperature ranges. For metals, toxicity may increase with either an increase or decrease in ambient temperature (Hodgson 1980). Temperature also affects the response of vegetation to air pollution. Generally, plant sensitivity to oxidants increases with increasing temperature up to 30°C. Soybeans are more sensitive to O_3 when grown at 28°C, regardless of exposure temperature or O_3 doses (Dunning et al. 1974).

5.5.2 pH

Maintenance of a particular pH in body fluids is critical for the well-being of animals and humans. The influence of pH on the toxicity of chemical agents varies according to the organism and the chemical agent. For instance, human body fluids must be maintained at a value near pH 7.4 for the body's metabolism to proceed properly. This is because most body enzymes function best when the pH remains around neutral. As noted in Chapter 4, the availability of metals in soil to plants varies most markedly with soil pH. Increases in acidity enhance the mobilization of metals in soil. Acid precipitation therefore may greatly increase the availability of toxic metals, such as aluminum, to plants.

5.5.3 Humidity

The sensitivity of plants to air pollutants increases with increase in relative humidity. For instance, high relative humidity has been shown to contribute to acute damage to forest vegetation by SO_2 (Linzon 1973). Injurious effects of O_3 and nitrogen dioxide (NO_2) on vegetation have also been found to be greater when the relative humidity is high. A similar effect was observed with fluoride. Gladiolus plants exhibited a higher sensitivity to fluoride when relative humidity increased from 50% to 80% (MacLean et al. 1973).

5.6 INTERACTION

The actions of individual toxicants are affected by many factors, such as portals of entry, mode, metabolism, and others described previously. However, organisms are generally exposed to a complex mixture of different pollutants. Simultaneous exposure to more than one toxicant can have a dramatic impact on the outcome of exposure. Toxicants may interact with each other to produce additive, potentiation, synergistic, or antagonistic effects. The factors affecting the outcome of exposure are complex and include the characteristics of the chemicals and the physiological condition of the organism, for example.

5.6.1 Additive, Synergism, and Potentiation Effects

An additive effect occurs when the combined effects of two compounds are simply additive. Synergism, however, describes a combined toxicity that is greater than the simple additive effects of two compounds. Potentiation means synergism in which one compound is generally assumed to have little or no intrinsic toxicity by itself, but when another compound is present, it adds its effect or the effect is increased. It should be noted that in synergism, both compounds can induce toxic effects.

Smoking and exposure to asbestos, for example, have been known to exhibit a synergistic effect, increasing lung cancer. The presence of particulate matter such as sodium chloride (NaCl) and SO_2, or SO_2 and sulfuric acid mist simultaneously, would have potentiation or synergistic effects on animals. Many insecticides exhibit synergism or potentiation. Studies with female rats showed that when the animals

TABLE 5.1
Synergistic Effect of O_3 and SO_2 on Plant Leaves

Duration (h)	Concentration (ppm) O_3	Concentration (ppm) SO_2	Leaf Damage (%)
2	0.03	0	0
2	0	0.24	0
2	0.031	0.24	38

Source: Menser, H.A., and H.E. Heggestad. Ozone and sulfur dioxide synergism. Injury to tobacco plants. *Science* 153, 424, 1955.

were exposed to fluoride and benzene hexachloride (BHC) simultaneously, a synergistic effect occurred, resulting in decreased red blood cell count, and relative weight of the ovary (Ramesh et al. 1997).

Exposure of plants to both O_3 and SO_2 simultaneously is more injurious than exposure to either of these gases alone. Laboratory studies showed that a single exposure to O_3 at 0.03 ppm and to SO_2 at 0.24 ppm for 2 hours or 4 hours did not injure tobacco leaves. However, when the leaves were exposed to a mixture of O_3 at 0.031 ppm and SO_2 at 0.24 ppm for 2 hours, a moderate (38%) injury to the older leaves of Tobacco Wel W3 occurred (Table 5.1) (Menser and Heggestad 1966). Similarly, an additive effect was observed on yield depression of bush beans grown in culture solutions containing 2×10^{-4} *M* cadmium (Cd) and 2×10^{-5} *M* nickel (Ni), while synergistic effects on yield depression were observed with the plants grown in culture solution containing 5×10^{-5} *M* zinc (Zn), 3×10^{-5} *M* copper (Cu), and 2×10^{-5} *M* nickel (Wallace and Romney 1977).

5.6.2 ANTAGONISM

Antagonism refers to a situation in which the toxicity of two or more chemicals present (or administered in combination or sequentially) is less than would be expected were the chemicals administered separately. Antagonism may be due to chemical or physical characteristics of the pollutants, or it may be due to the biological actions of the chemicals involved. For example, the highly toxic metal cadmium is known to induce anemia and nephrogenic hypertension, as well as teratogenesis, in animals. Zinc and selenium (Se) act to antagonize the action of cadmium. This appears to be due to zinc and selenium inhibiting the renal retention of cadmium.

Antagonism includes cases for which the lowered toxicity is caused by inhibition or induction of detoxifying enzymes. For example, parathion is known to inhibit mixed-function oxidase (MFO) activity, while DDT and dieldrin are inducers. The induction of MFO activity may also protect an animal from the effect of carcinogens by increasing the rate of detoxification. Antagonistic effects on xenobiotic metabolism in vivo are also known in humans. Cigarette smoking affects the activities of various liver enzymes. For instance, studies on the term placenta of smoking mothers have shown that smoking causes marked stimulation of aryl hydrocarbon hydroxylase and related activities. Physical means of antagonism can also exist. For example, oil mists have been shown to decrease the toxic effects of O_3 and NO_2 or certain hydrocarbons in experiments on mice. This may be due to the oil dissolving the gas and holding it in solution or to the oil containing neutralizing antioxidants.

5.7 BIOLOGICAL FACTORS

5.7.1 PLANTS

Plants exhibit marked differences in their susceptibility to environmental pollutants. Genetic variation is probably the most important factor affecting plant response. The response varies between species of a given genus and between varieties within a given species. Such variation is a function of the influence genetic variability has on morphological, physiological, and biochemical characteristics of plants. For instance, gladiolus is known to be extremely sensitive to fluoride, and different gladiolus varieties show different responses to fluoride. The susceptibility of different species of plants to different pollutants varies markedly. For example, DDT applied to soil at a rate of 50 ng/g inhibited germination, seedling height, and fresh and dry weight in oilseed plants, but had no effect on rice, barley, and mung bean plants. In the oilseed plants, DDT exposure caused a reduction in cell number and length and inhibition of ion uptake, especially potassium ions (K^+) and calcium ions (Ca^{2+}) (Mitra et al. 1991).

It has been shown that the sensitivity of two onion cultivars to O_3 is controlled by a single gene pair. After exposure to O_3, the stomata of an O_3-resistant cultivar were found to be closed, with no appreciable injury, whereas the stomata of O_3-sensitive cultivar remained open, with obvious injury (Engle and Gabelman 1966). The sensitivity of plants to air pollutants is also affected by leaf maturity. Generally, young tissues are more sensitive to peroxyacyl nitrate (PAN) and hydrogen sulfide (H_2S), and maturing leaves are most sensitive to the other airborne pollutants. According to Linzon (1973), in white pine the greatest chronic injury occurred in second-year needles exposed to SO_2.

5.7.2 ANIMALS AND HUMANS

Genetics, development, health status, behavior, and gender are among the most important factors that affect the response of animals and humans to pollutant toxicity (Hodgson 1980).

5.7.2.1 Genetic Factors

Not all organisms, including humans, react in the same way to a given dose of an environmental pollutant. In animal experiments, variation between species, as well as variation between strains within the same species, occurs. As shown in Table 5.2, the toxicity of the insecticides DDT and dieldrin differs markedly for different species. Substantial epidemiological data exist to illustrate that the interplay between

TABLE 5.2
Toxicity of DDT and Dieldrin

Compound	Organism	LD_{50} (mg/kg body weight)
DDT	Housefly	8
DDT	Bee	114
Dieldrin	Housefly	1.3
Dieldrin	Rat	87

environmental and genetic factors in the development of congenital malformations is significant. In animals, for example, it has often been shown that the rates of congenital anomalies differ between different strains of mice in response to the same dose of a teratogen (Finnell et al. 2002).

In humans, such factors as serum, red blood cells, immunological disorders, and malabsorption can contribute to differences in individuals' response to stresses caused by environmental pollutants. For example, individuals with sickle-cell anemia are more susceptible to the effect of toxicants than are individuals without the anemia. People with malabsorptive disorders may also have problems; they may suffer nutritional deficiencies, which in turn may lead to an increased susceptibility to toxicants.

5.7.2.2 Developmental Factors

The developmental factor category includes aging, immature immune system, pregnancy, immature detoxification systems, and circadian rhythms. Examples of these factors, which all contribute to the varied responses to xenobiotics exhibited by individuals, include decline in renal function as a result of aging; lack of γ-globulin needed to cope with invading bacteria and viruses; lack of receptors needed in hormonal action; greater stresses encountered by pregnant women when metabolizing and detoxifying xenobiotics (not only for themselves but also for their fetuses); and an immature hepatic MFO system in the young.

5.7.2.3 Diseases

Diseases in lungs, heart, kidneys, and liver predispose a person to more severe consequences of pollutant exposure. As mentioned previously, these organs are responsible for metabolism, storage, and excretion of environmental pollutants. Cardiovascular and respiratory diseases of other origins decrease an individual's ability to withstand superimposed stresses. An impaired renal function will certainly affect the ability of the kidneys to excrete toxic substances or their metabolites. As noted, the liver plays a vital role in detoxification of chemicals in addition to its role in the metabolism of different nutrients and drugs. Disorders in the liver can therefore disrupt proper detoxification processes.

5.7.2.4 Behavioral Factors

Smoking, drinking, and drug habits are some examples of lifestyle choices that can affect an individual's response to toxicants. Smoking has been shown to act synergistically with several environmental pollutants. Asbestos workers or uranium miners who smoke are known to have higher lung cancer death rates than those who do not smoke. Heavy drinking can lead to disorders in the brain and liver; a heavy drinker exposed to certain organic chemicals may therefore experience more serious liver injury than would a nondrinker.

5.7.2.5 Gender

The rate of metabolism of foreign compounds varies in animals and humans according to gender. The response to $CHC1_3$ exposure by laboratory mice, for example, shows a distinct sex variation. Male mice are highly sensitive to $CHC1_3$, and death often results following their exposure to this chemical (M.H. Yu, unpublished data, 2004). The higher sensitivity exhibited by male mice to certain toxicants may be due to their inability to metabolize the chemicals as efficiently as female mice. It is interesting that the death rate of male mice exposed to $CHC1_3$ is also dependent on the strain of mouse. Studies have shown that the effect of BHC on the weights of the brains and kidneys of rats varied with sex of the animal. The brain and kidney weights did not differ in male rats exposed to 25 ppm BHC from those of unexposed controls, but in female rats, the weights of brain and kidney were both increased.

5.8 NUTRITIONAL FACTORS

5.8.1 Introduction

Results obtained from human epidemiological and animal experimental studies have clearly shown nutrition as an important factor affecting pollutant toxicity. For example, human populations exposed to environmental fluoride may or may not exhibit characteristic fluoride poisoning depending on their nutritional status, such as the adequacy of protein or vitamins A, C, D, or E. The interaction between nutrition and environmental pollutants is complex, and its study is a challenge to researchers in the fields of toxicology and nutrition; a new area of study called nutritional toxicology has emerged in recent years.

The relationship between nutrition and toxicology may include the effect of nutritional status on the toxicity of environmental chemicals, the additional nutritional demands as a result of toxicant exposure, and the presence of toxic substances in foods (Parke and Loannides 1981). Generally, nutritional modulation can alter rates of absorption of environmental chemicals and therefore affect the circulating levels of those chemicals. Nutrition modulation can also induce changes in body composition, which in turn may result in altered tissue distribution of chemicals. Dietary factors can also influence renal function and pH of body fluids with altered toxicity. In addition, modified nutritional status of an individual may alter the responsiveness of the target organ.

5.8.2 Fasting and Starvation

Fasting or starvation, the most severe forms of nutritional modulation, influences the toxicity of xenobiotics in such a way that they may cause depressed metabolism and so reduced clearance of chemical agents. Consequently, increased toxicity may be seen.

Studies with animals have shown that the effect of fasting on microsomal oxidase activity is species, substrate, and sex dependent. For instance, some reactions are decreased in male rats but increased in female rats, while others may not be affected at all. It is thought that the sex-dependent effect is related to the ability of androgen to enhance binding of some substrates to cytochrome P450. Animal studies also showed that glucuronide conjugation was decreased under starvation.

5.8.3 PROTEINS

The effects of proteins on the toxicity of environmental chemicals include both quantitative and qualitative aspects. Laboratory animals fed low-protein diets and exposed to toxicants often show higher toxic effects than observed in animals fed normal-protein diets. Protein deficiency causes hypoproteinemia and impaired hepatic function, leading to decreased levels of hepatic proteins, DNA, and microsomal P450, as well as lowered plasma binding of xenobiotics. Plasma contains many different proteins, such as albumin, glycoprotein, and lipoprotein. Albumin, in particular, has an important role in the binding and distribution of xenobiotics in the body, so lowered binding of xenobiotics by plasma albumin could result in greater toxicity.

Protein deprivation may impair the metabolism of toxicants that occur in the body. It has been known for a long time that protein deficiency increases the toxicity of chemical compounds and drugs. The toxicity of most pesticides, such as chlorinated hydrocarbons, herbicides, fungicides, and acetylcholinesterase (AChE) inhibitors, is increased by protein deficiency (Table 5.3). Studies by Tandon et al. (1998) showed that the activities of the antioxidant enzymes including SOD, glutathione peroxidase (GSHPx), and catalase, were decreased in rats fed a low-protein diet (containing 8% protein). In addition, the rats showed significantly increased levels of lipid peroxidation. Alteration of xenobiotic metabolism by protein deprivation may lead to either enhanced or decreased toxicity, depending on whether the metabolites are more or less toxic than the parent compounds. The results shown in Table 5.3 reveal that low-protein diets can cause decreased metabolism but increased mortality with respect to the chemicals concerned. In contrast, rats treated under the same conditions showed a decrease in mortality with respect to heptachlor, $CC1_4$, and aflatoxin B1 (AFB1), a toxin produced by *Aspergillus flavus*. It is known that heptachlor and AFB1 are metabolized in the liver to their respective epoxide forms (Figures 5.3 and 5.4, respectively) that are more toxic than the parent substances. For example, the epoxide form of AFB1 (AFB1 epoxide) produces DNA adducts by binding to guanine (Jones et al. 1999). As mentioned in Chapter 4 (Reaction 4.5), $CC1_4$ is metabolized to a highly reactive free radical $\cdot CCl_3$.

Although protein nutrition has a critical influence on pollutant toxicity, it should be recognized that severely limited protein intake in humans is usually accompanied by inadequate intake of other nutrients. Hence, it is often difficult to identify specific pathological conditions merely associated with protein deficiency.

5.8.4 CARBOHYDRATES

A high-carbohydrate diet usually leads to a decreased rate of detoxification. Microsomal oxidation is generally depressed when the carbohydrate/protein ratio is

TABLE 5.3
Effect of Protein on Pesticide Toxicity[a]

	LD$_{50}$ (mg/kg body weight)	
	3.5% Casein Diet	26% Casein Diet
Chlorinated Hydrocarbons		
DDT	45	481
Chlordane	137	217
Toxwaphene	80	293
Endrin	6.69	16.6
Organophosphates		
Parathione	4.86	37.1
Marathione	759	1,401
Herbicides and Fungicides		
Diuron	437	2,390
Captan	480	12,600

Source: Tandon, A., D.K. Dhawan, and J.P. Nagpaul. Effect of lithium on hepatic lipid peroxidation and antioxidative enzymes under different dietary protein regimens. *Appl. Toxicol.* 18, 187, 1998.

[a] Male rats fed for 28 days from weaning on diets of varying casein contents.

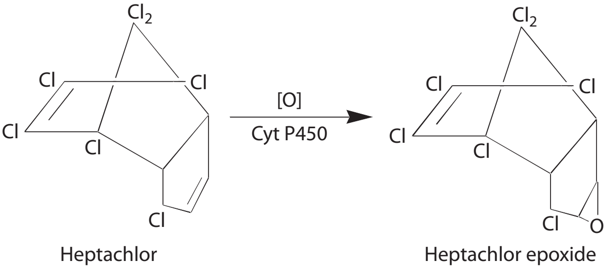

Cl₂
Cl
Cl
Cl
Cl
Cl
[O]
Cyt P450
Cl₂
Cl
Cl
Cl
Cl
Cl
O
Heptachlor
Heptachlor epoxide

FIGURE 5.3 Formation of heptachlor epoxide.

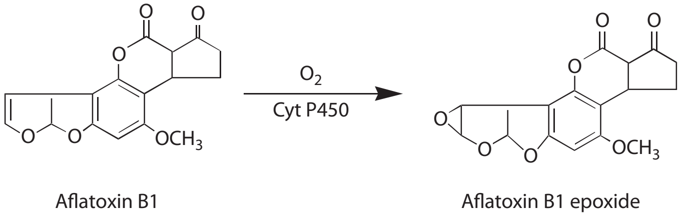

O
O
O
O
O
OCH₃
O₂
Cyt P450
O
O
O
O
O
O
OCH₃
Aflatoxin B1
Aflatoxin B1 epoxide

FIGURE 5.4 Formation of aflatoxin B1 (AFB1) epoxide.

increased. In addition, the nature of the carbohydrates also affects oxidase activity. For example, sucrose gives rise to the lowest activity, cornstarch gives the highest value, and glucose and fructose give intermediate values. Since dietary carbohydrates influence body lipid composition, the relationship between carbohydrate nutrition and toxicity is often difficult to assess. However, environmental chemicals can affect, or be affected by, body glucose homeostasis in several different ways. For example, $CC1_4$ rapidly deactivates hepatic glucose 6-phosphatase by damaging the membrane environment of the enzyme. Trichloroethylene and several other compounds that are metabolized by the liver to glucuronyl conjugates are more hepatotoxic to fasted animals than fed animals.

5.8.5 LIPIDS

Dietary lipids may affect the toxicity of environmental chemicals by delaying or enhancing their absorption. The absorption of lipophilic substances is accelerated, while that of lipophobic substances delayed. The endoplasmic reticulum contains high levels of lipids, especially phospholipids (which are rich in polyunsaturated fatty acids), Lipids may influence the detoxification process by affecting the cytochrome P450 system because phosphatidylcholine is an essential component of the hepatic microsomal MFO system. A high-fat diet may cause more oxidation to occur because it may contribute to more incorporation of membrane material.

The type of lipid can also affect toxicant metabolism, as a high proportion of phospholipids is unsaturated due to the presence of linoleic acid (18:2) in the (3-position of triacylglycerol). Dietary 18:2 is important in determining the normal levels of hepatic cytochrome P450 concentration and the rate of oxidative demethylation in rat liver.

Dietary lipids play a unique role in the toxicity of chlorinated hydrocarbon pesticides. Dietary lipids may favor more absorption of these pesticides, but once these chemicals are absorbed into the body, they may be stored in the adipose tissue without manifestation of toxicity. For this reason, obesity in humans is considered protective against chronic toxicity of these chemicals. Similarly, the body fat in a well-fed animal is known to store organochlorine pesticides. Fat mammals, fish, and birds are thus more resistant to DDT poisoning than their thinner counterparts. In times of food deprivation, however, organic chemicals, such as DDT and PCB, may be mobilized from their fat deposits and reach concentrations potentially toxic to the animal.

A report released by the U.S. Institute of Medicine (IOM) stating the need to reduce saturated fat intake among the population as a means of reducing exposure to dioxins raises another concern about the toxicants. The report pointed out that saturated fats are a key source of human exposure to dioxins. Dioxins are a collection of more than 200 related compounds that may be linked to hormonal changes, neurodevelopmental problems in children, cancer, and other adverse effects. Dioxins are ubiquitous agents that contaminate food as they cycle through the biosphere. Because dioxins are lipid soluble, they accumulate in many varieties of foods. According to the IOM, saturated fats in meat, dairy products, and certain species of fish are the biggest sources of human exposure to these chemicals (Schmidt 2004).

The role of dietary lipids in affecting pollutant toxicity has been fairly well defined for a few specific chemicals, including lead (Pb), fluoride, and hydrocarbon carcinogens. For example, high-fat diets are known to increase lead absorption and retention. Moreover, competitive absorption of lead and calcium (Ca) also occurs, which is probably due to competition for the calcium-binding protein (CaBP) whose synthesis is mediated by vitamin D, a fat-soluble vitamin. Studies have shown that a high-fat diet causes increased body burden of fluoride, resulting in higher toxicity. This is attributed to the delay of gastric emptying caused by high-fat levels. Consequently, enhanced fluoride absorption may occur, leading to increased body burden of fluoride. Dietary fat does not increase metabolic toxicity of fluoride itself, however. As is well known, AFB1 (Figure 5.4) is a potent liver cancer-causing agent. A high-fat diet offers protection from lethal effects of the toxin, presumably through dissolution of the carcinogen.

5.8.6 Vitamin A

Many reports describe vitamin A and its synthetic analogues as a potential factor in the prevention and treatment of some cancers. There is growing evidence that vitamin A may also alleviate pollutant toxicity. Epidemiological studies using a cohort of 8,000 men showed a low incidence of lung cancer in those with high dietary vitamin A intake, while incidence was higher in individuals with a diet low in the vitamin. In experimental studies, rats exposed to PCB, DDT, and dieldrin showed a 50% reduction in the liver vitamin A store. In other studies, rats deficient in vitamin A exhibited lowered cytochrome P450 activity in the liver. The effect of vitamin A deficiency on MFO enzymes, however, depends on several factors, such as substrate, tissue, and animal species. Recent studies have shown that rats exposed to fluoride showed increased levels of lipid peroxide (LPO) in the liver, serum, heart, and kidneys, whereas the activities of SOD and GSHPx and the levels of GSH (glutathione) were decreased. Administration of β-carotene (which can be partially converted to vitamin A in the body) reduced LPO levels while increasing SOD activity (Sun 1998).

Some epidemiological studies suggested that people who eat foods rich in β-carotene and vitamin A are less likely to develop various types of cancer, especially lung cancer. But when researchers tested β-carotene supplements in smokers, they found that people who took the supplements were more likely to develop lung cancer. These findings led the experts to advise people—especially former and current smokers—not to take β-carotene (Harvard Medical School Special Health Report, 2010).

The mechanism involved in vitamin A action relative to carcinogenesis may in part involve a free-radical scavenging action of the vitamin. Because vitamin A is required in the differentiation of epithelial cells, which are important in both respiratory and gastrointestinal tracts, its deficiency may affect transformation of epithelia and thus predispose the tissue to neoplastic changes.

5.8.7 Vitamin D

The role that vitamin D plays in the prevention of rickets and osteomalacia has been well documented. To play its role in the maintenance of calcium homeostasis, vitamin

D must be converted into its metabolically active form, 1,25-dihydroxy-D_3 (the hormone-like substance). In this case, vitamin D_3 (cholecalciferol) is first hydroxylated in the liver to 25-hydroxy-D_3. The resultant 25-hydroxy-D_3 is then converted in the kidney to 1,25-dihydroxy-D_3, the active form of the vitamin. The 25-hydroxylation of cholecalciferol requires NADPH (nicotinamide adenine dinucleotide phosphate), O_2, and an enzyme whose properties are similar to those of microsomal MFO (Bjorkhelm et al. 1979). In addition, 25-hydroxy-D_3 has been shown to competitively inhibit some cytochrome P450 reactions in vitro. Patients suffering from drug-induced osteomalacia show increased rates of catabolism of vitamin D_3 to 25-hydroxy-D_3. In a laboratory study of male mice exposed to NaF, vitamin D, alone or in combination with vitamin E, was found to ameliorate the adverse effect of NaF on reproductive function and fertility (Chinoy and Sharma 1998).

Some studies have shown that vitamin D can reduce the risk of heart disease. The studies showed that raising the amount of vitamin D in the blood appears to help some people, at least those deficient in the vitamin, reducing their risk of heart disease by about 30%. Recent studies showed that as many as three-quarters of Americans have a concentration in their blood that is under the normal level of 30 ng/ml (Harvard Medical School Special Health Report 2010).

Vitamin D appears to protect against colon cancer in some studies. For example, a cross-sectional study of 3,121 adults ages 50 and older found that those with highest vitamin D intakes (>645 IU/day) were less likely to have cancerous lesions than those with lower intakes (Harvard Medical School Special Health Report, 2010).

5.8.8 VITAMIN E (α-TOCOPHEROL)

Vitamin E, a membrane-bound antioxidant and free-radical scavenger, appears to offer protection against injuries caused by O_2, O_3, and NO_2 and nitrosamine formation. Male rats administered daily doses of 100 mg α-tocopherol acetate and exposed to 1.0 ppm O_3 were shown to survive longer than rats deficient in vitamin E. The action of O_3 is attributed in part to formation of free radicals. Vitamin E is also believed to protect phospholipids of microsomal and mitochondrial membranes from peroxidative damage by reacting with free radicals (Figure 5.5). Because lipid peroxidation is associated with decrease in oxidase activities, it is expected that the enzyme activity is affected by dietary vitamin E. Maximum activity has been observed when diets include both polyunsaturated fatty acids and vitamin E.

Nitrosamine, known to be carcinogenic, leads to liver cancer. The interaction between vitamin E and nitrosamines is attributed to the inhibitory effect of the vitamin on nitrosamine formation; that is, vitamin E competes for nitrite, a reactant in nitrosamine formation.

Laboratory studies with isolated rat hepatocytes showed that cellular α-tocopherol maintains the viability of the cell during a toxic insult (Fariss et al. 1985). A recent study showed that male mice treated with NaF (10 mg fluorine per kg body weight) exhibited changes in epididymal milieu, as revealed by significant decreases in levels of sialic acid and protein and ATPase (adenosine triphosphatase) activity in epididymides. These changes in turn disrupted the sperm maturation process, leading to a significant decline in cauda epididymal sperm count, motility, and viability.

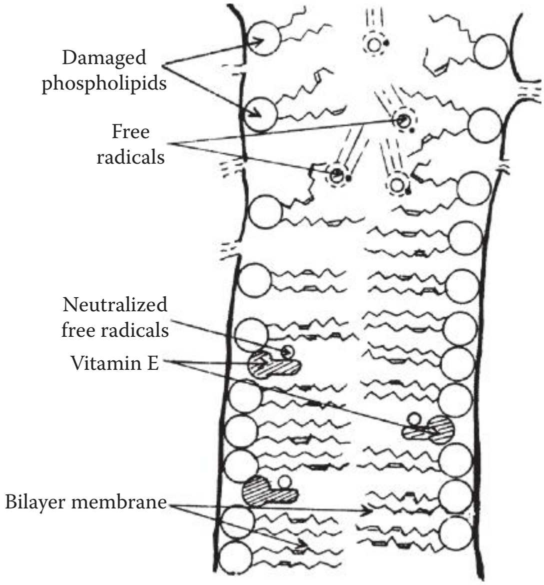


FIGURE 5.5 Action of vitamin E to stop free-radical-induced chain reactions in microsomal and mitochondrial membrane.

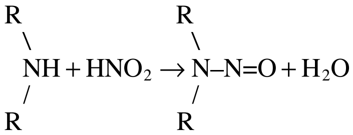
Consequently, a significant decline in fertility rate occurred. Withdrawal of NaF treatment for 30 days produced incomplete recovery. However, vitamin E supplementation during the withdrawal period resulted in recovery of all NaF-induced adverse effects (Kuenzig 1977).

5.8.9 VITAMIN C

Vitamin C (ascorbic acid) is found in varying amounts in almost all animal and human body tissues. In humans, high vitamin C levels occur particularly in adrenal and pituitary glands, eye lens, and various soft tissues. Vitamin C is a potent antioxidant and participates in many cellular oxidation-reduction reactions. Vitamin C-deficient guinea pigs have been shown to exhibit an overall deficiency in drug oxidation, with marked decreases in N- and O-demethylations and in the contents of cytochrome P450 and cytochrome P450 reductase (M.H. Yu, unpublished data, 2004). Administration of ascorbate to the deficient animals for 6 days reversed these losses of MFO activity.

The effect of vitamin C appears to be tissue dependent (Kuenzig 1977). Epidemiological studies show that persons with high intakes of dietary vitamin C or citrus fruit have a lower-than-normal risk of developing cancer. Cancer prevention by vitamin C is thought to be mainly due to its role as an antioxidant and free-radical scavenger. Oxidative and free-radical-induced damage to DNA and cell membranes has been considered as the most important factor in cancer initiation; substantial evidence indicates that vitamin C can help prevent such damage (Block 1992).

A variety of experimental tumors of the gastrointestinal tract, liver, lung, and bladder can be produced by nitroso compounds (Narisawa et al., 1976; Mirvish et al, 1975). Nitroso compounds are produced by the reaction of nitrite with secondary and tertiary amines, amides, or others, as shown in Reaction 5.1:



$$\begin{array}{lcl} \mathrm{R} & & \mathrm{R} \\ \;\backslash & & \;\backslash \\ \;\;\mathrm{NH} + \mathrm{HNO_2} & \rightarrow & \mathrm{N{-}N{=}O} + \mathrm{H_2O} \\ \;/ & & \;/ \\ \mathrm{R} & & \mathrm{R} \end{array} \qquad (5.1)$$

The nitrosation of several secondary and tertiary amines can be blocked in vitro by the addition of vitamin C. The vitamin appears to compete for the nitrite, thus inhibiting nitrosation. It has been demonstrated that vitamin C does not react with amines or enhance the rate of nitrosamine decomposition. However, it reacts rapidly with nitrite and nitrous acid. The vitamin appears to decrease the available nitrite by reducing nitrous acid to nitric oxide (Reaction 5.2), leading to inhibition of the nitrosation reaction.

$$\text{Ascorbate} + 2\mathrm{HNO_2} \rightarrow \text{Dehydroascorbate} + 2\mathrm{NO} + 2\mathrm{H_2O} \qquad (5.2)$$

Vitamin C has been shown to prevent growth retardation and severe anemia in young Japanese quail exposed to cadmium (Fox and Fry 1970). Vitamin C, with vitamin E, has been shown also to protect against herbicide-induced lipid peroxidation in higher plants. Cell damage is markedly increased in plants that have much lower or much higher than normal ratio of vitamin C to vitamin E concentrations (10 to 15:1, w/w) or a lower amount of both vitamins (Finckh and Kunert 1985).

The average American is thought to ingest approximately 70 μg cadmium, 0.9 μg arsenic, and 4.1 mg nitrite per day, as well as being exposed to ambient air containing CO, O_3, lead, cigarette smoke, and other materials (Calabrese 1980). In view of the many vital functions that vitamin C performs in biological systems, and of the increasing exposure of people to various drugs and xenobiotics, some researchers have suggested that the recommended dietary allowances (RDAs) for vitamin C may be inadequate (Zannoni 1977). In support of the suggestion is the result of a study on urban air pollution. The study showed that short-term exposure produced some decrease in lung function, which might be counteracted by pretreatment with vitamin C (Bucca et al. 1992).

In a separate study on mice, fluoride was shown to impair the protective enzymes, including SOD, GSHPx, and catalase, thereby increasing ovarian LPO and injury. Vitamins C and E were shown to ameliorate the detrimental effects induced by fluoride (Chinoy and Patel 1998).

The most outstanding chemical characteristics of the ascorbate system (ascorbic acid/ascorbate, ascorbate free radical, dehydroascorbic acid) are its redox properties. Ascorbate is a reactive reductant, but its free radical (A^-) is relatively nonreactive. Interestingly, there is evidence that vitamins E and C probably act synergistically; that is, vitamin E acts as the primary antioxidant (particularly in biomembranes), and the resulting vitamin E radical (E·) then reacts with ascorbate (AH^-) to regenerate vitamin E (Rielski 1982), as shown in Reaction 5.3.

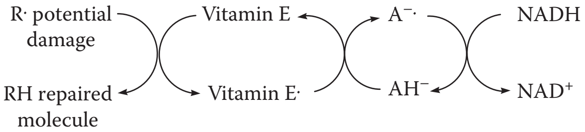


FIGURE 5.6 Interaction of vitamin C (ascorbate, AH^- with vitamin E).

$$\text{Vitamin E}\cdot + AH^- \rightarrow \text{Vitamin E} + A\cdot^- \tag{5.3}$$

The interaction between vitamin E radicals and ascorbate in protecting against potentially damaging organic free radicals is illustrated in Figure 5.6.

5.8.10 MINERALS

Mineral nutrition influences toxicology in different ways. Interactions are the rule rather than the exception when considering the effects of trace nutrients on detoxification. As with the macronutrients, trace mineral elements can influence absorption of xenobiotics. Divalent cations can compete for chelation sites (Figure 5.7) in intestinal contents, as well as for binding sites on transport proteins. It is widely known that competitive absorption of lead and calcium occurs, which is probably due to competition for binding sites on intestinal mucosal proteins mediated by vitamin D. However, zinc is known to provide protection against cadmium and lead toxicities (Sandstead 1980). Absorption of zinc is facilitated by complexing with picolinic acid, a metabolite of the amino acid tryptophan. Although both cadmium and lead form complexes with picolinic acid, the resulting complexes are less stable than the zinc complex. Selenium is antagonistic to both cadmium and mercury, thus reducing their toxicity. In addition, selenium enhances vitamin E function in the prevention of lipid peroxidation. The mechanisms involved in the functioning of selenium and vitamin E are, however, different. Whereas α-tocopherol functions as a membrane-bound antioxidant, acting as a free-radical scavenger, selenium participates at the active site of GSHPx and is thus part of the enzyme. GSHPx protects membrane lipids by catalyzing the destruction of H_2O_2 and organic hydroperoxides before they cause membrane disruption.

Since cytochrome P450 requires iron (Fe) for its biosynthesis, deficiency of iron may lead to depressed MFO activity. Dietary iron deficiency in rats has been shown to result in a rapid loss of the cytochrome P450 content and MFO activity in the villous

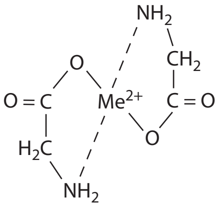


FIGURE 5.7 Example of chelation.

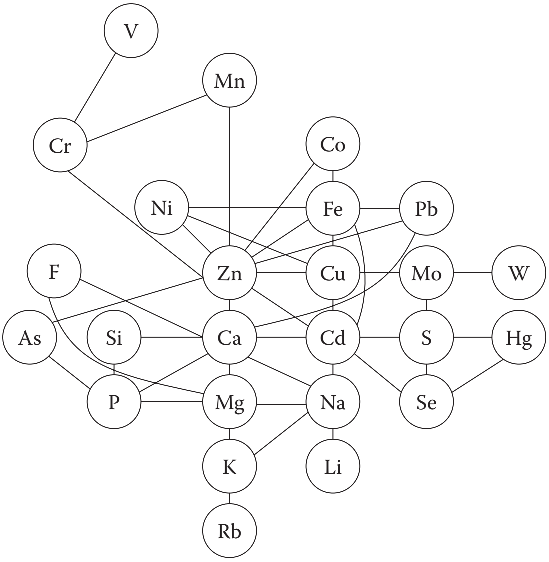


FIGURE 5.8 Interactions among mineral elements.

cells of duodenal mucosa (Hoensch et al. 1975). As noted, rats fed a low-protein diet exhibited increased levels (56%) of LPO and decreased activities of antioxidant enzymes, such as SOD, GSHPx, and catalase. When lithium (Li) (as carbonate) was administered to rats fed a low-protein diet, the activity of GSHPx was increased, while the activities of catalase and SOD were brought to within normal limits. Furthermore, lithium treatment diminished the increase in LPO level (Tandon et al. 1998).

Dietary magnesium (Mg) and potassium (K) restriction has been shown to enhance the toxicity of paraquat (an organic herbicide) in rats (Minakata et al. 1998). The main mechanism involved in paraquat toxicity is tissue oxidation by reactive oxygen radicals generated by redox cycling of the compound (Bus and Gibson 1984). Rats fed a magnesium-restricted diet and exposed to paraquat exhibited a severe toxicosis, whereas those with a potassium-restricted diet showed a mild toxicosis. Restriction of magnesium and potassium was shown to have a synergistic effect on paraquat-dependent toxicosis (Hoensch et al. 1975). Figure 5.8 shows the interaction among mineral elements.

REVIEW QUESTIONS

1. Explain how protein nutrition may affect the body's response to environmental toxicants.
2. What is meant by oxygen stress?
3. What are antioxidants? Give four examples of both endogenous antioxidants and antioxidant enzyme systems.
4. Explain how vitamins C and E act as free-radical scavengers. What are the main differences between these two vitamins when they act as free-radical scavengers?

5. Which is generally more injurious to an organism exposed to a toxicant, a continuous exposure or an intermittent exposure?
6. Explain the differences between synergism and antagonism.
7. Explain how zinc and cadmium may interact.
8. What are the reasons for old and young people to be more susceptible than adults to toxicant-induced injury?
9. How may nutrition generally affect toxicology?
10. What role do dietary lipids play in affecting the toxicity of organochlorine pesticides?
11. Explain the relationship between vitamin A and fluoride-induced toxicity.
12. Explain the role that vitamin E plays in lipid peroxidation.
13. What role do vitamins C and E play in nitrosation?
14. Why is iron deficiency related to the MFO system?
15. Explain the relationship between a low-protein diet and the levels of antioxidant enzymes.
16. How does ascorbate interact with vitamin E free radicals?
17. What role does selenium play in detoxification? What is the mechanism involved in such a reaction?

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6 Biotransformation
Metabolism of Xenobiotics

6.1 INTRODUCTION

Biotransformation refers to chemical alteration of a substance within the body, as by the action of enzymes. *Metabolism*, on the other hand, is the sum of all chemical reactions that occur within a living cell. The purpose of cellular metabolism is to maintain the homeostasis of the cell within a population of other cells. *Homeostasis* refers to a tendency toward maintenance of a relatively stable internal environment in the bodies of higher animals through a series of interacting physiological processes. Metabolism is usually subdivided into two categories: *anabolism* and *catabolism*. Anabolism is the synthesis of larger molecules from smaller ones. The synthesis of protein from its amino acid building blocks is an example. Anabolism generally requires input of energy from an energy source, such as ATP (adenosine triphosphate). Catabolism, on the other hand, refers to the degradation of larger molecules to smaller ones (e.g., the breakdown of starch to glucose). In higher organisms, catabolism of carbohydrates and fats results in the production of ATP.

Following their absorption into a mammal, xenobiotics are subjected to metabolic conversion in the body, resulting in structural changes. This metabolic process is called biotransformation. Biotransformation may occur in any of several body tissues and organs, including skin, lung, intestine, liver, and kidney. The liver carries out the majority of the chemical reactions because it contains a large number of nonspecific enzymes capable of biotransformation of xenobiotics. The enzymes involved in the biotransformation are named *mixed-function oxidase* (MFO), commonly known as cytochrome P450. The liver metabolizes not only many xenobiotics, but also drugs to which the body is exposed. Biotransformation in the liver is thus a critical process in the body's defense against the toxic effects of a wide variety of xenobiotics (Kappas and Alvares 1975).

6.2 TYPES OF BIOTRANSFORMATION

As mentioned in Chapter 4, the process of xenobiotic biotransformation consists of two phases: phase I and phase II (Figure 6.1). Phase I includes oxidation, reduction, and hydrolysis, whereas phase II is essentially composed of conjugation reactions.

Among the representative oxidation reactions catalyzed by cytochrome P450 are hydroxylation of an aliphatic or aromatic carbon; dealkylation, including atoms such as oxygen (O), sulfur (S), or nitrogen (N); deamination; epoxidation; oxidative group transfer; and dehydrogenation. Occasionally, hydroxylation is treated as an inde-

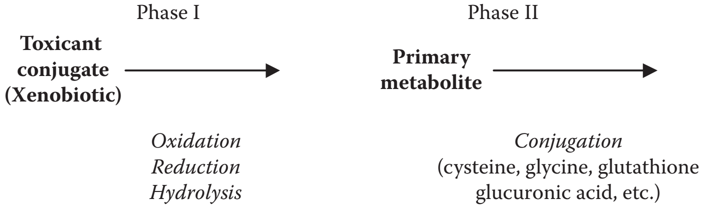


FIGURE 6.1 The two phases of biotransformation.

pendent reaction system. Dealkylation produces an aldehyde, whereas deamination produces ammonia or an amine and the primary metabolite (Figure 6.2).

Xenobiotics containing an aldehyde, ketone, disulfide, sufoxide, azo, nitro group, as well as certain metals or metalloids, are often reduced in vivo. Several endogenous reducing agents are involved in the reduction processes. They include the reduced forms of glutathione (GSH), flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (phosphate) (NAD[P]). Figure 6.2 shows examples of azo-reduction and nitro-reduction.

In hydrolysis, splitting of ester and amide bonds is common. Hydrolytic enzymes contained in mammals include carboxylesterases, cholinesterases, and organophosphatases. These are involved in hydrolyzing functional groups, such as carboxylic acid ester, phosphoric acid ester, and acid anhydride (Figure 6.2).

6.3 MECHANISM OF BIOTRANSFORMATION

In the two phases of biotransformation, a lipophilic foreign chemical is first oxidized in a phase I reaction so that a functional group, such as –OH, $–NH_2$, –COOH, or –SH, is introduced into the substrate, forming a product called a primary metabolite. A slight increase in hydrophilicity usually occurs as a result of the reaction.

Phase II reactions, on the other hand, are a synthesis of conjugation processes. Here, a primary metabolite from phase I biotransformation, or a parent xenobiotic, reacts with an endogenous substance and forms a conjugate (Figure 6.2). Included in this process are sulfation, acetylation, methylation, glucuronidation, and conjugation with GSH or amino acids. Most phase II biotransformation results in substantial increase in xenobiotic hydrophilicity, thus facilitating the excretion of xenobiotics. Many xenobiotics are lipophilic and undergo phase I and phase II reactions sequentially, whereas others may participate in only one phase. In the latter case, a toxicant may combine directly with an endogenous substance, forming a conjugate. Endogenous substances known to participate only in phase II reactions include glycine, cysteine, GSH, glucuronic acid, sulfates, and some other water-soluble substances. Several representative phase II reactions are shown in Figure 6.3.

6.4 CHARACTERISTICS OF BIOTRANSFORMATION

The NADPH-cytochrome P450 system, commonly known as the *mixed-function oxygenase* system (*MFO system*), is the most important enzyme system involved in phase I biotransformation. The cytochrome P450 system, localized in the smooth

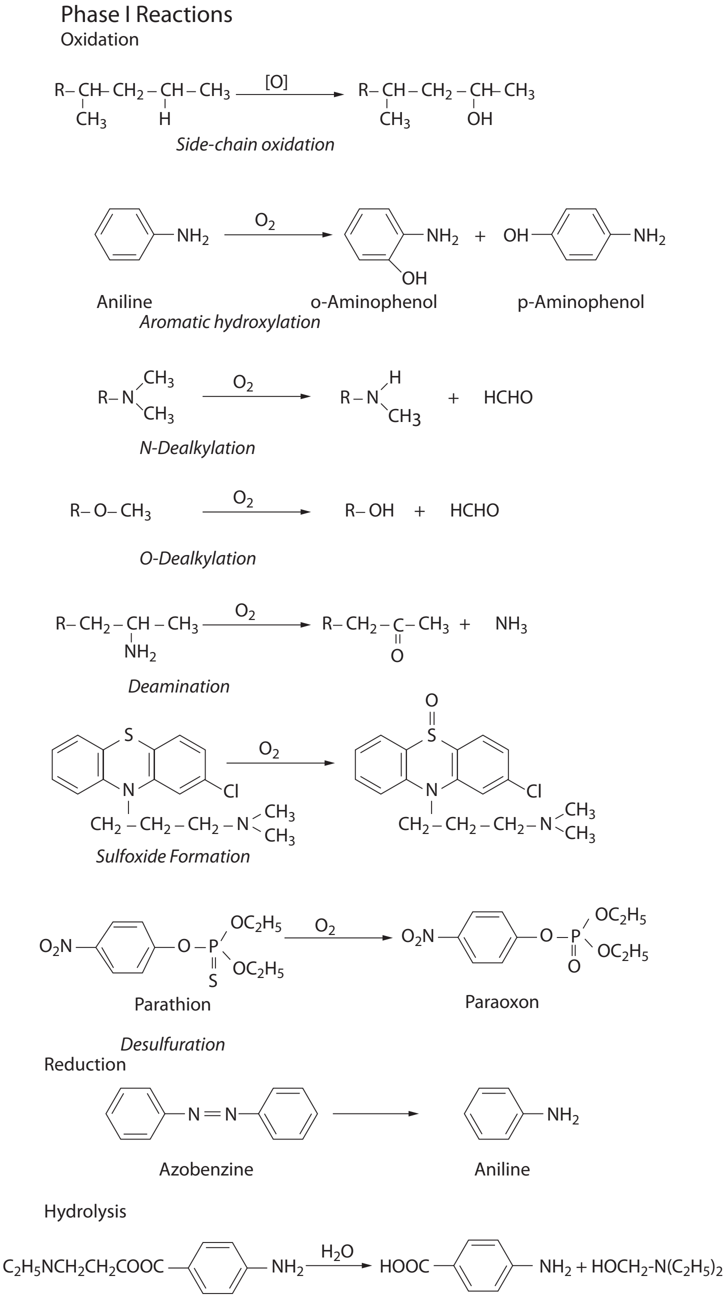

Phase I Reactions
Oxidation
R–CH–CH2–CH–CH3
CH3
H
[O]
R–CH–CH2–CH–CH3
CH3
OH
Side-chain oxidation
NH2
O2
NH2
OH
+
OH
NH2
Aniline
o-Aminophenol
p-Aminophenol
Aromatic hydroxylation
R–N
CH3
CH3
O2
R–N
H
CH3
+
HCHO
N-Dealkylation
R–O–CH3
O2
R–OH
+
HCHO
O-Dealkylation
R–CH2–CH–CH3
NH2
O2
R–CH2–C–CH3
O
+
NH3
Deamination
S
N
Cl
CH2–CH2–CH2–N
CH3
CH3
O2
O
S
N
Cl
CH2–CH2–CH2–N
CH3
CH3
Sulfoxide Formation
O2N
O–P
OC2H5
OC2H5
S
O2
O2N
O–P
OC2H5
OC2H5
O
Parathion
Paraoxon
Desulfuration
Reduction
N=N
NH2
Azobenzine
Aniline
Hydrolysis
C2H5NCH2CH2COOC
NH2
H2O
HOOC
NH2 + HOCH2-N(C2H5)2

FIGURE 6.2 Examples of phase I biotransformation reactions.

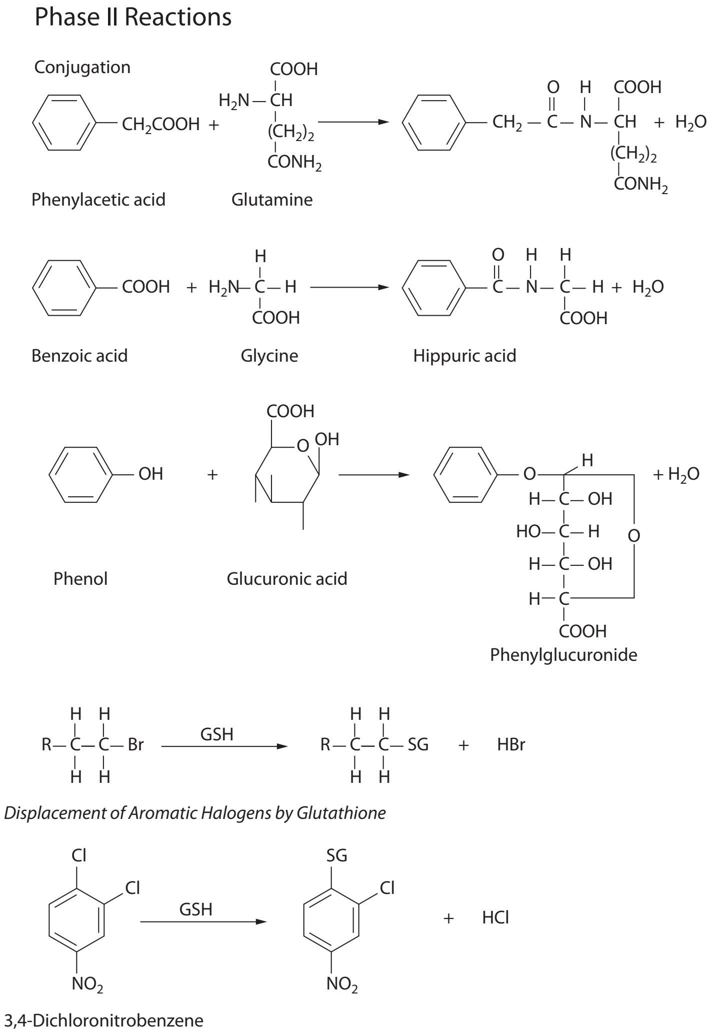

Phase II Reactions
Conjugation
Phenylacetic acid
Glutamine
Benzoic acid
Glycine
Hippuric acid
Phenol
Glucuronic acid
Phenylglucuronide
GSH
Displacement of Aromatic Halogens by Glutathione
GSH
3,4-Dichloronitrobenzene

FIGURE 6.3 Examples of phase II biotransformation reactions.

endoplasmic reticulum of cells of most mammalian tissues, is particularly abundant in the liver. Contrary to most enzymes, which catalyze the metabolism of one substrate with one mechanism, quickly and efficiently, the cytochrome P450 system contains a number of isozymes—multiforms of an enzyme that are structurally equivalent but catalytically distinct from one another—that can catalyze a variety of substrates with multiple mechanisms, slowly and inefficiently (average turnover rate is one per minute). The reactions that the isozymes catalyze include aliphatic or aromatic hydroxylation, epoxidation of a double bond, N-oxidation, sulfoxidation, dealkylation, deamination, dehydrogenation, dehalogenations, oxidative transfer, and cleavage of esters (Figure 6.2) (Wislocki et al. 1980).

During the catalytic reaction, the oxidized form of an iron atom (Fe^{3+}) at the active site of cytochrome P450 binds directly to the substrate (XH). Reduction of this enzyme-substrate complex follows, with an electron being transferred from NADPH via NADPH cytochrome P450 reductase. The reduced (Fe^{2+}) enzyme-substrate complex binds molecular oxygen (O_2) and is reduced further by a second electron (presumably donated by NADH [nicotinamide adenine dinucleotide] via cytochrome b5 reductase). The enzyme-substrate-oxygen complex splits into oxidized substrate, water, and the oxidized form of the enzyme. The overall reaction by which a substrate or an environmental chemical, XH, is oxidized by the cytochrome P450 system is shown in Reaction 6.1:

$$\text{XH (substrate)} + O_2 + \text{NADPH} + H^+ \rightarrow \text{XOH (product)} + H_2O + \text{NADP}^+ \quad (6.1)$$

As shown in Reaction 6.1, one atom of oxygen from the molecule of O_2 is reduced to water, and the other is incorporated into the substrate, producing ROH, a hydroxylated metabolite. The constituents required in this enzyme system are O_2, NADPH, and magnesium ions (Mg^{2+}).

Carbon monoxide (CO) readily binds the reduced form of the cytochrome, forming a complex with a maximum absorption at 450 nm. (This is the origin of the name of the enzyme cytochrome P450.) Formation of the CO complex causes the inhibition of enzyme activity and thus the oxidation processes.

Unlike the cytochrome P450 system, most hepatic phase II enzymes are located in the cytoplasmic matrix. For the biotransformations to proceed properly, each of the participating enzymes must function efficiently. It is also obvious that sufficient intracellular content of cofactors is required for one or more reactions. Required cofactors include NADH, NADPH, O_2, glucose 1-phosphate, glucuronate, ATP, cysteine, and GSH.

6.5 CONSEQUENCE OF BIOTRANSFORMATION

Xenobiotics in a biological system are removed primarily by biotransformation and excretion mechanisms. Some xenobiotics, especially the lipophilic ones, are readily reabsorbed by the kidney cells. Unless the chemicals are converted to more polar metabolites, they will remain in the body, mostly in the fatty tissues, for a long period. As described in Section 6.3, the resultant products from biotransformation are usually, but not always, more hydrophilic or polar than the parent substance and thus more readily excreted.

6.5.1 Biotransformation of Endogenous Substances

Although hepatic enzymes that catalyze biotransformation are responsible for the conversion of xenobiotics, they also participate in the catabolism, or breakdown, of endogenous substances. For example, the hormone testosterone is deactivated by cytochrome P450. The S-methylases detoxify hydrogen sulfide (H_2S) formed by anaerobic bacteria in the intestinal tract. It follows that chemicals or conditions that influence the activity of phase I and phase II enzymes can affect the normal metabolism of endogenous substances.

6.5.2 ACTIVATION OF XENOBIOTICS

Although the biotransformation of lipophilic xenobiotics often results in the production of a more stable, water-soluble, and more readily excretable metabolite, the activation of xenobiotics also occurs. In other words, certain xenobiotics may be converted through biotransformation to reactive electrophilic species that are more potent than the parent compounds. For example, benzo[a]pyrene (BaP), which is both mutagenic and carcinogenic, is biotransformed to BaP-epoxide and BaP-7,8-diol before forming its final product BaP-7,8-dihydrodiole-9,10-epoxide (Figure 6.4a). This compound is more toxic than BaP itself because it can readily combine with guanine in DNA to form an adduct. Similarly, aflatoxin B1 is toxic because its metabolite aflatoxin B1 epoxide can cause liver cancer (see Section 5.8.3). Another example is carbon tetrachloride (CCl_4). This substance is hepatotoxic because, after it is metabolized, it yields trichloromethyl free radical (Reaction 4.5), which binds to protein and initiates lipid peroxidation (Figure 6.4b).

The reactive chemical species produced during biotransformation must be metabolized; otherwise, they may interact with a nucleophilic site and a vital cell constituent and induce cellular damage (Reynolds 1977). As mentioned, many of the reactive metabolites can bind covalently to macromolecules in liver cells. For instance, the hepatotoxic CCl_4 can covalently bind to lipid components of the liver endoplasmic reticulum (Reynolds and Moslen 1980). Some of the reactive electrophiles are also carcinogenic.

Although liver cells depend on detoxification enzymes for protection against the reactive electrophilic species produced during biotransformation, endogenous

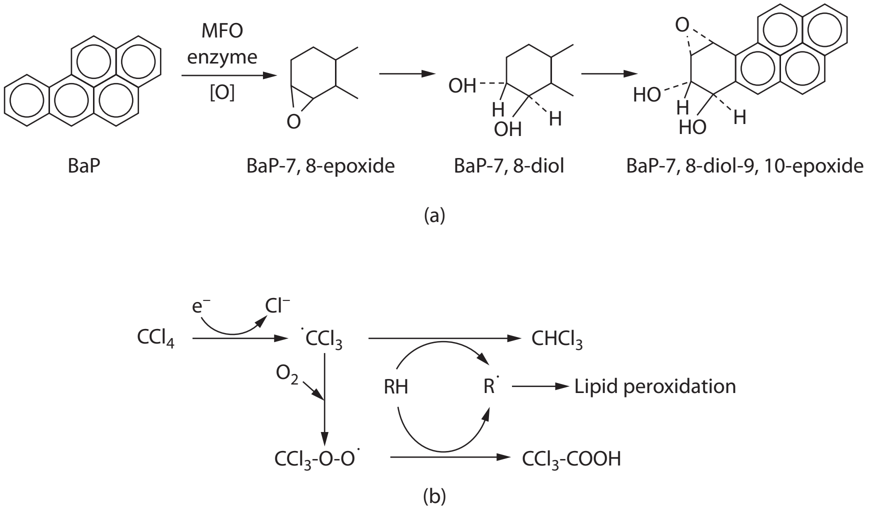
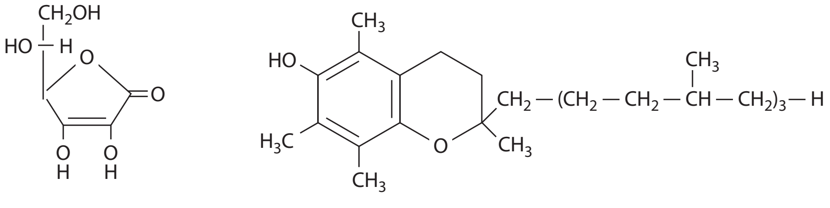
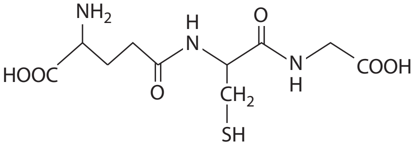


FIGURE 6.4 Activation of xenobiotics through biotransformation: (a) benzo[a]pyrene; (b) CCl_4.



Ascorbic acid (vitamin C)

Vitamin E



Glutathione (GSH)

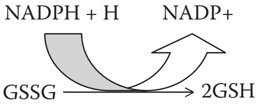
FIGURE 6.5 Examples of antioxidant chemical species.

antioxidants, such as vitamin E (α-tocopherol) and tripeptide GSH (L-γ-glutamyl-cysteinyl-glycine) (Figure 6.5), also provide protection. Vitamin E is widely known as a free-radical scavenger. Its main role is to protect lipid material in membranes against free-radical-initiated peroxidation reactions (see Sections 4.4 and 5.8). Experimental evidence indicates that livers of animals fed diets deficient in vitamin E were more vulnerable to lipid peroxidation following exposure to CCl_4 than those fed diets containing supplemental vitamin E (Reynolds and Moslen 1980). GSH, conversely, has a nucleophilic sulfhydryl (–SH) group (Figure 6.5) that can react with, and thus detoxify, reactive electrophilic species (Van Bladeren et al. 1980). GSH can also donate its sulfhydryl hydrogen to a reactive free radical. The resultant GSH radical (GS·) can react with another GS·, producing a molecule of stable glutathione disulfide (GSSG) (Reactions 6.2 and 6.3).

$$\mathrm{GSH} + \mathrm{X}\cdot \rightarrow \mathrm{HX} + \mathrm{GS}\cdot \tag{6.2}$$

$$\mathrm{GS}\cdot + \mathrm{GS}\cdot \rightarrow \mathrm{GSSG} \tag{6.3}$$

The resultant GSSG can be reduced back to GSH through a NADPH-dependent reaction catalyzed by glutathione reductase (Reaction 6.4). The NADPH is derived from reactions in the pentose phosphate pathway.



(6.4)

In addition to antioxidant chemical species, such as vitamins E and C and GSH, there are several enzymes, called *antioxidant enzymes*, that play a pivotal role in the defense

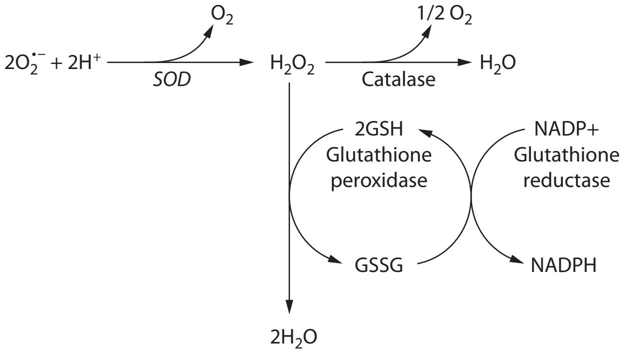


FIGURE 6.6 Examples of reactions involving antioxidant enzymes.

against free-radical-mediated cellular damage. These include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), and glutathione reductase. Figure 6.6 shows the interrelationship between these enzymatic components.

6.6 FACTORS AFFECTING BIOTRANSFORMATION

The activity and levels of each of the P450 enzymes have been shown to vary between individuals, depending on environmental and genetic factors. Increased P450 enzyme activity can result from exposure to xenobiotics or other environmental factors that induce the synthesis of the enzyme, stimulation of preexisting enzyme by a xenobiotic, or gene duplication leading to overexpression of a P450 enzyme. Decreased P450 enzyme expression or a mutation leads either to blocking the synthesis of a P450 enzyme or to synthesis of an enzyme with limited activity or total inactivity (Parkinson 2001).

For example, in animals many drugs and environmental chemicals can either stimulate or inhibit microsomal enzymes, resulting in stimulation or inhibition of biotransformation. Chemical agents that can stimulate enzymes include halogenated hydrocarbon insecticides, urea herbicides, polycyclic aromatic hydrocarbons (PAHs), nicotine and other alkaloids, and some food preservatives. Agents that can inhibit the enzymes include not only CO, as mentioned, but also ozone (O_3), CCl_4, and organophosphorus insecticides. The consequences of the actions of these chemicals on microsomal enzymes is complex and may include normal body constituents such as steroid hormones, thyroxin, and bilirubin or altered metabolism and action of drugs and carcinogens.

The health status of an individual, including nutritional status, also plays an important role in biotransformation. As noted, several endogenous substances, such as glycine, glutamine, glucuronic acid, and GSH, are necessary for conjugation with xenobiotics or their metabolism in phase II biotransformation (Figure 6.3). Glycine and glutamine are both amino acids, GSH is a tripeptide derived from three amino acids, and glucuronic acid is formed from glucose that is derived directly from a dietary source or from liver glycogen through glycogenolysis (breakdown of glycogen). It is therefore likely that the availability of proteins or liver reserves of glycogen may be insufficient in individuals with poor nutritional status or liver problems. These conditions can lead to impaired biotransformation.

The importance of ethoxyresorufin O-deethylase (EROD), one of the hepatic cytochrome P450-dependent monooxygenases, has become widely known and is increasingly accepted as an indicator of exposure to common organic pollutants. Various organic chemicals, including chlorinated hydrocarbons, have been shown to adversely affect the enzymes involved in biotransformation. This was manifested in a study summarized in Case Study 6.1. The results suggest that pollutants such as polychlorinated biphenyls (PCBs) may activate detoxification capacity, but weaken antioxidant status, in fish in a polluted river system (Otto and Moon 1996).

Case Study 6.1

A group of researchers collected brown bullheads (*Amerurus nebutosus*) from the St. Lawrence River, a relatively polluted system, and compared various parameters in the fish with those in fish from Lac La Peche, a relatively nonpolluted system in Canada (used as reference) (Otto and Moon 1996). The main results obtained were as follows:

- The activities of liver EROD—a common phase I enzyme—in fish collected from the St. Lawrence River were significantly higher (2.8-fold) than those in fish from the reference site.
- The conjugation activity by hepatic glutathione S-transferase (GST) was three times higher in fish from the St. Lawrence River than in fish from the reference site.
- The content of PCBs in white muscle was 22 times higher in fish from the St. Lawrence River than in fish from the reference site.
- The activities of cytosolic SOD were significantly higher, while those of CAT in kidney and GSHPx in red and white muscles were lower in the St. Lawrence River fish than in the reference fish.
- The concentrations of total GSH in different tissues were significantly lower in liver, kidney, and white muscle of fish from the St. Lawrence River compared with those in fish from the reference site.

The important role that SOD plays in biotransformation is well recognized. Several factors can either enhance or inhibit the enzyme, fluoride being one of them. For example, both in vivo and in vitro studies showed that the activity of SOD from the earthworm (*Eisenia fitida*) was stimulated by NaF in a concentration-dependent manner (Lawson and Yu 2003). Other studies showed that, in laboratory animals exposed to NaF, the activities of both SOD and GSHPx decreased significantly (Sun et al. 1994). However, in aluminum plant workers exposed to fluoride and other air pollutants, serum SOD activity was shown to be enhanced (Sun et al. 1997). The reason for this discrepancy is not clear.

6.7 CHARACTERISTICS OF THE CYTOCHROME P450s

As noted, cytochrome P450s consists of a number of isozymes. Researchers have divided the isozymes into several categories, such as CYP1, CYP2, and CYP3. These

TABLE 6.1
Characteristics of Human Cytochrome P450 Enzymes

Cytochrome P450 (CYP)	Subfamily	Characteristics
CYP1	CYP1A1	Found in human lung, skin, intestine, lymphocytes, and placenta (induced in the lungs of smokers); can activate such xynobiotics as benzo[a]pyrene and other polycyclic aromatic hydrocarbons
	CYP1A2	Found in human liver; important in drug metabolism; thought to be responsible for metabolic activation of polycyclic aromatic hydrocarbons, aromatic amines, and nitrosamines (e.g., acetaminophen, 2-acetylaminofluorene, naphthylamine)
CYP2	CYP2D6	Found in human liver; important in drug metabolism; primarily metabolizes hydrophobic amines; possible link between rapid metabolizers and lung cancer
	CYP2E1	Important in metabolism of a large number of halogenated alkanes; involved in certain carcinogenesis activities, such as those involving nitrosamines, acrylonitrile; benzene, carbon tetrachloride, chloroform, ethyl carbamate, trichloroethylene, and vinyl chloride
CYP3	CYP3A4	Found in liver, small intestine, and kidney; inducible by glucocorticoids and phenobarbitol; important in drug metabolism; metabolizes a wide variety of hydrophobic substances, including activation of aflatoxin B1, nitroaromatics, cyclophosphamide, etc.
	CYP3A5	Found in placenta; expressed in liver in 15% of the population, but in 80% of all human kidneys; substrate specificity similar to CTP3A4
	CYP3A7	Found in fetal liver; not found in adults except in placenta; metabolizes dehydroepiandrosterone sulfate

are further divided into subfamilies according to their properties. The nomenclature and major characteristics of the cytochrome P450 isozymes are shown in Table 6.1.

6.7.1 INDUCTION

One of the characteristics of cytochrome P450s is that a number of the isozymes are inducible on exposure to xenobiotics or some drugs (Table 6.1). For instance, CYP1A1 and CYP1A2 are induced in smokers, while CYP2E1 is induced by ethanol, isoniazid, and so on. Studies have shown that MFO enzymes were increased in organisms after they were exposed to xenobiotics. Such inducers of cytochrome P450 increase the rate of xenobiotic biotransformation. As a consequence of this phenomenon, it would be expected that there could be increases in the activation of procarcinogens to DNA-reactive metabolites, leading to increased tumor formation. However, it is not clear whether this is indeed the case in humans. In many instances, P450 induction does not necessarily enhance the biotransformation of the inducer (Parkinson 2001).

Fish from waters that receive pulp-mill effluents have been shown to respond to the effluents with increases in hepatic MFO activity, particularly that of EROD (Munkittrick et al. 1994). In one study, a compound isolated from a bleached kraft mill effluent (tentatively identified as a chlorinated pterostilbene) was shown to be capable of causing MFO induction in rainbow trout and in a hepatocyte cell line (Burnison et al. 1999).

6.7.2 Genetic Polymorphisms

Another characteristic feature of cytochrome P450s is the occurrence of genetic polymorphism, resulting in enzyme levels and activities varying greatly between different individuals. Genetic polymorphism includes defects in CYP2D6 and variations in CYP1A2 activities. For example, three phenotypes are found in CYP1A2: slow, medium, and rapid metabolizers. Research shows that this phenomenon is related to susceptibility of individuals to certain types of cancer, for example, increased bladder and colorectal cancer susceptibility for rapid 1A2 populations. Similarly, marked ethnic differences exist with CYP2D6, and usually 1% to 10% of the population are poor metabolizers who may develop less-aggressive forms of bladder cancer. Conversely, a possible link appears to exist between rapid metabolizers and lung cancer (Korzekwa 1994). Several P450s are also involved in steroid biosynthesis and metabolism. As mentioned, some researchers suggest that a general increase in estrogenic activity may be responsible for the observed increase in breast and testicular cancers.

REVIEW QUESTIONS

1. What is the significance of biotransformation in the body's response to environmental chemicals?
2. What are the main differences between phase I and phase II reactions?
3. Give the three types of reactions in phase I biotransformation.
4. List the names of functional groups that participate in phase I biotransformation.
5. List the characteristics of the mixed-function oxidase (MFO).
6. What specific role does the liver play in biotransformation?
7. List the endogenous substances that are associated with phase II reactions.
8. What are the possible problems involved in biotransformation?
9. Give an overall reaction whereby an environmental chemical, RH, is oxidized by the cytochrome P450 system.
10. Name the four major antioxidant enzymes.
11. Give the names of cellular antioxidants that may prevent free-radical-mediated cellular damage.
12. Explain the important role that SOD plays in the cell.
13. Which environmental chemicals can inhibit microsomal enzymes?
14. List four environmental chemicals that can stimulate microsomal enzymes.
15. What is meant by inducibility of cytochrome P450 system?
16. Briefly explain the term *genetic polymorphism.*
17. Explain how cytochrome P450s may be related to cancer.

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7 Responses to Environmental Toxicants

7.1 INTRODUCTION

From our previous discussion, it is clear that living organisms are exposed to different types of environmental toxicants. These organisms also take in a number of essential nutrients from their diet or exterior environment, and through various processes, they store, translocate, or excrete some of these substances. On the other hand, living organisms often possess certain defense mechanisms to cope with the actions of toxicants to which they are exposed. This chapter discusses some of these processes.

7.2 RESPONSES OF PLANTS

Several physiological and biochemical mechanisms occur in plants that may protect them against the toxic effects of pollutants absorbed into the tissue. For example, onion has been shown to vary in sensitivity to O_3 depending on its cultivars. Engle and Gabelman (1966) showed that, following exposure to O_3, the stomata of the resistant cultivar of the onion were closed with no appreciable injury, whereas the stomata of the sensitive cultivar remained open, manifesting obvious injury.

Studies have also shown that when plants were exposed to heavy metals, particularly cadmium (Cd) or lead (Pb), they produced a kind of polypeptide called phytochelatins. The polypeptides in phytochelatins have been shown to be rich in sulfur. The general structure of the compounds is depicted as $(\text{Glu-Cys})_n$-Gly, where the three amino acids refer to glutamate, cysteine, and glycine, respectively, while n refers to a number from 2 to 11. The SH group contained in cysteine can bind covalently to heavy metals, as discussed in Chapter 4.

A variety of free radicals is produced naturally in cellular metabolism. For example, superoxide radicals can be formed by γ-ray irradiation along with their dismutation products singlet oxygen and hydrogen peroxide (Van Hemmen and Meuling 1975). As discussed in Chapter 6, several cellular antioxidants occur to counteract the action of these free radicals. In addition, endogenous antioxidants, such as vitamins C and E and glutathione (GSH), and antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GSHPx), and GSH reductase all help counteract the free radicals.

Laboratory experiments with plant tissues showed that the activity of SOD is enhanced in tissues exposed to low concentrations (0.1 m*M*) of NaF, while it was inhibited in tissues exposed to high concentrations of NaF (Wilde and Yu 1998). Laboratory studies also showed that SOD occurred in the earthworm and that NaF inhibited its activity (Lawson and Yu 2003).

7.3 RESPONSES OF HUMANS AND ANIMALS

This section focuses on responses of four body systems: the respiratory tract, membranes, liver, and kidneys in humans and animals, whenever applicable.

7.3.1 The Respiratory Tract

An adult breathes in more than 13,000 L of air a day. This is not only the body's largest intake of any substances but also air is the most immediately important gas to life. Humans can go on without food for many days and without water for many hours without causing serious health effects, but life without air will terminate in a very few minutes. Air is inhaled through the nasal cavity, nasopharynx, and trachea. The trachea divides into the main *bronchi*, which extend to the right and left lungs (Figure 7.1). The left lung consists of two lobes, while the right lung has three. The bronchi divide into finer and finer tubes, called *bronchioles*. Located at the ends of the bronchioles are many tiny air sacs called *alveoli*, where the gas exchange takes place. At the alveoli, a thin sheet of moving blood picks up molecular oxygen (O_2) from the inhaled air and unloads CO_2 for exhalation. The respiratory tract is one of the principal ports of entry for air pollutants and is remarkably well equipped to cope with harmful invaders. There are three main processes that operate in their defense against the invasion of foreign agents: filtration, inactivation, and removal.

7.3.1.1 Nasopharynx

Air that is drawn in through the nose and the upper throat is warmed and moistened as it moves to the lungs. Particulate matter is likewise moistened as it enters the nose. Large particles are filtered and removed by the hair at the entrance of the nose, while larger-size particles such as dust, carbon, and pollen spores are washed out with the aid of mucus.

7.3.1.2 Tracheobronchial Areas

The response of the tracheobronchial area to the large particulates is contraction of the muscles, causing the lumen of the bronchi to narrow. This results in removal of solid particulate matter with a diameter above 5 mm, permitting less of the particulate matter to enter the lower portion of bronchial tubes. The mucus that is secreted moistens the particulates, and as they accumulate, they are removed through the cough reflex. Spasm—involuntary muscular contraction—of the bronchi may be induced, and this tends to prevent invading agents from reaching the air sacs, but this can also lead to respiratory distress. An important feature of the trachea is the action of cilia, the hairlike structures that beat rhythmically back and forth in the air passage (Figure 7.1a). With a speed of 1,300 beats per minute, billions of these cilia function like a broom to sweep noxious foreign agents out of the system.

The condition commonly called bronchitis is due to infection of the air passages beginning with the nose and extending through the bronchioles. Acute bronchitis may result from inhaled irritants such as smoke, dust, and chemicals. It can also be due to allergy. Chronic bronchitis usually develops slowly and appears in people past

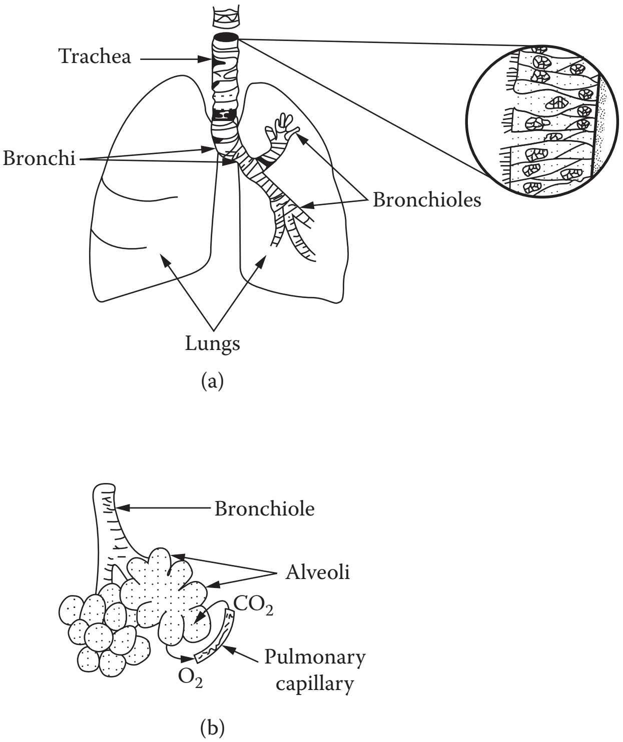

FIGURE 7.1 Generalized structure of human lungs: (a) the tracheobronchial area, with microscopic view showing a section of the ciliated epithelium that lines the passages (inset), and (b) alveoli.

life's midway point. It occurs about four times more often in men than women and more often among city dwellers than rural residents. The most significant symptom is cough, which may be constant or intermittent. Mucus is almost always coughed up, which may be clear or may contain pus or streaks of blood. Since the patient is not severely ill or incapacitated, in many cases medical help is not sought, and cough and expectoration persist.

7.3.1.3 Alveoli

Particulate matter that reaches the alveoli (Figure 7.1(b)) and is deposited there is usually 1 µm in diameter or less. Particulates with a diameter less than 0.5 mm are small enough to behave like gases. There are about 400 million air sacs in the lungs of a healthy adult. The inner surfaces of the alveoli, continuous with the bronchioles, bronchi, and trachea, are technically outside the body since they are in contact with the atmosphere. If the walls of all the air cells were spread out as one continuous area, they would cover a surface the size of a tennis court. Because this immense surface is compacted into the small space of our two lungs, the walls of the air cells are extremely thin. This is essential to allow absorption of O_2 from air and dispersal of CO_2 waste gas (Figure 7.1(b)). Particulate matter that reaches the alveoli and is deposited is usually 1 μm or less in diameter. Particulates with a diameter less than 0.5 μm are small enough to behave like gases.

There are four types of cells in the alveoli: alveolar epithelial cells, endothelial cells, large alveolar epithelial cells, and alveolar macrophages. Alveolar epithelial cells are responsible for the exchange of CO_2 and O_2; alveolar endothelial cells are endowed with various protective properties; and large alveolar cells and alveolar macrophages carry out oxidative and synthetic processes that defend the lungs against invading organic and inorganic materials.

Macrophages play a well-known phagocytic role in the lungs and other tissues. They engulf an organism or a particle by membrane invagination and pouch formation and are one of the most important components of the immune response. A number of environmental agents, such as silica, asbestos, cigarette smoke, carbon monoxide (CO), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), formaldehyde, and aflatoxin and other mycotoxins, can either depress or enhance the phagocytic function of macrophages.

The term *emphysema*, derived from Greek words meaning "overinflated," refers to a condition in which the structures of the alveoli are overinflated. Tiny bronchioles through which air flows to and from the air sacs have muscle fibers in their walls. In an emphysematous patient, the structures of bronchioles and air sacs may become hypertrophied and lose elasticity. Air will flow into the air sacs easily but cannot flow out easily because of the narrowed diameter of the bronchioles. The patient can breathe in but cannot breathe out efficiently, resulting in too much stale air in the lungs. As pressure builds up in the air cells, their thin walls are stretched to the point of rupture, so several air spaces communicate and the area of the surfaces where gas exchange takes place is decreased. Figure 7.2 illustrates the comparison between a healthy person and an emphysematous patient in their alveoli and the volume of exhaled air.

Smog, smoke, and inhaled irritants may increase mucus secretion in the air passages and cause obstruction of bronchioles, with entrapment of air beyond obstruction. The result is shortness of breath, overwork of the heart, and sometimes death. Some studies associate emphysema with smog, particularly NO_2, O_3, SO_2, and heavy cigarette smoking.

7.3.2 MEMBRANES

Both the plasma and intracellular membranes of mammalian cells have similar overall compositions: about 60% protein and 40% lipid by weight. In addition, some membranes contain small amounts of carbohydrates as glycoproteins or glycolipids. The human erythrocyte membrane, for example, contains about 10% carbohydrates, which appear to be localized on the outer surface of the membranes.

The basic chemical components of the membrane are phospholipid bilayer and protein. Phospholipids are the major structural components of lipid bilayers. Structurally, the phospholipid bilayer is embedded with protein complexes, and it is this characteristic that provides the permeability barrier of the cell. The phospholipid bilayer consists mainly of phosphatidyl choline, phosphatidyl ethanolamine,

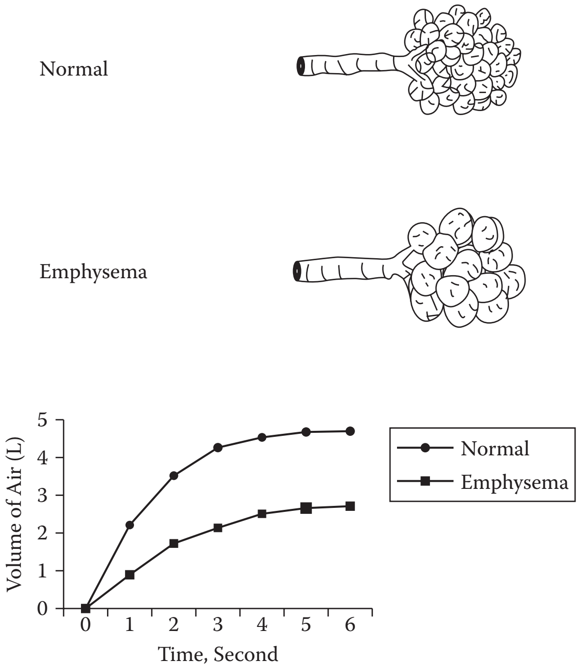

FIGURE 7.2 Effects of emphysema on lung function. Normal: Lung surface area is normal; volume of exhaled air is normal. Emphysema: Decrease in lung surface area due to overexpansion of alveoli and reduction in exhaled air volume.

sphingomyelin, and phosphatidyl serine. The other major lipid is cholesterol. All phospholipids are composed of two hydrophobic hydrocarbon chains linked to a charged polar head group via the glycerol backbone. Phospholipid bilayer membranes thus consist of a hydrophobic core, largely impermeable to water and other hydrophilic solutes, with polar surfaces that may or may not bear a net surface charge depending on the particular phospholipids. Membrane proteins are grouped into two categories: extrinsic and intrinsic proteins. Some of the membrane proteins are structural, but others are enzyme proteins such as ATPase (adenosine triphosphatase) and cytochrome oxidase.

The cell membranes serve as the major barrier to the absorption of foreign toxic compounds. The membranes may be those surrounding the cells of the skin or lining the gastrointestinal tract or those of the alveoli in the lung. The passage of a compound across the membranes is therefore an important factor contributing to absorption. In addition, membranous barriers influence translocation of any chemical from the exterior of a cell to the intracellular fluid of a cell within the animal. As mentioned in Chapter 4, a toxicant that gains entry by the mouth must pass from the gastrointestinal tract to the circulation and then to the cell. Such a process involves a series of translocation steps and increases the possibility of chemical exposure to large endogenous molecules, such as proteins, which may effectively bind and therefore functionally change and remove the offending chemical from the animal or humans.

On the other hand, certain chemicals may react with membrane material such as proteins and alter the structure of the membrane. For instance, heavy metals such as lead, cadmium, and mercury (Hg) may react with the –SH groups on the protein molecules in the membrane. Similarly, O_3 may induce peroxidation, thus altering the lipid constituent of the membrane, as mentioned previously. Furthermore, the free radicals formed in the reaction can attack not only lipids but also proteins and disrupt the membrane.

7.3.3 LIVER

The liver (Figure 7.3) is the largest solid organ of the body and is an incomparable chemical plant. As noted, the liver plays a foremost role in detoxifying xenobiotics. In addition, it is a blood reservoir and a storage organ for some vitamins and for digested carbohydrates as glycogen, which is broken down to release glucose to sustain blood sugar levels. The liver is also a manufacturing site for enzymes, cholesterol, proteins, vitamin A (from carotenoids), blood coagulation factors, and other elements.

Although the liver is noted for its ability to regenerate under different conditions, it can nevertheless be severely damaged. For example, *cirrhosis* (a chronic progressive disease of the liver that is characterized by an excessive formation of connective tissue followed by hardening and contraction, widely known to be related to alcoholism and poor nutrition) may be caused by chronic exposure to chemicals such as CCl_4. Another liver disease is fibrosis, which is characterized by the deposition of excessive amounts of collagen such that the features of the lobules are accented. Hepatic fibrosis can result from repeated exposure or continuous injury following prolonged low-level exposure to environmental chemicals. Portal fibrosis with portal hypertension has also been reported in humans exposed to arsenic (As) (Eisler 1994) compounds or vinyl chloride (Gedigk et al. 1975; Thomas and Popper 1975).

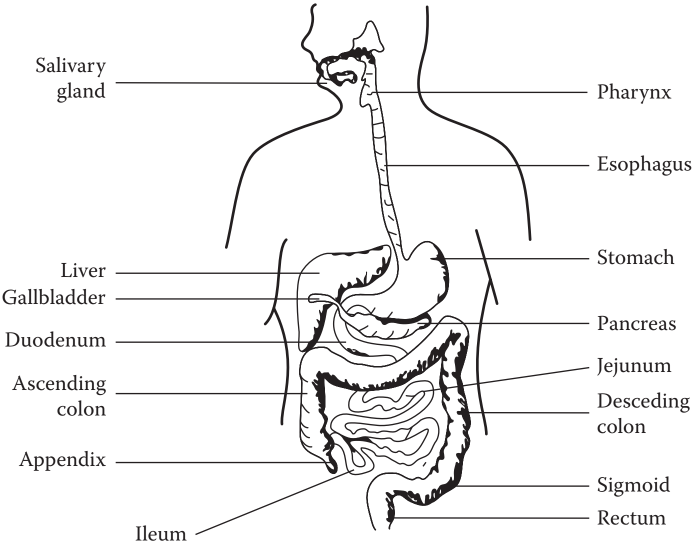

FIGURE 7.3 Human digestive system.

7.3.4 KIDNEYS

The kidneys are the principal organs for excretion of both endogenous and exogenous toxins. About one-fourth of the blood pumped by each stroke of the heart passes through the kidneys. Our kidneys incessantly filter various substances from the blood, reabsorb some of them, and concentrate wastes created by the chemical processes of living into urine to be excreted from the body. Optimal mechanisms for excretions depend on selective conservation of essential nutrients and their metabolites as well as on transport of toxins, thus reducing the potential for cell injury.

The urine-forming unit of the kidney is called the *nephron*. It is a microscopic filtration plant of exquisite design, consisting of several intricate structures, including the Bowman's capsule and the glomerulus. The glomerulus ("little ball"), a tufted network of intricately laced capillaries, is nested in the capsule and ends in a collecting tubule located toward central part of the kidney. Practically all the constituents of blood, except blood cells and most proteins, can pass from the capillaries into the space between the double walls of the capsule. The resulting filtrate contains many dissolved materials, some of which are indispensable for the body's welfare, while some others may be harmful.

The filtering process of the glomeruli is physical, not chemical. The area of the filtering surface of glomeruli of a single kidney is as large as the surface of the entire body, and the glomerular capillaries of both kidneys would stretch more than 35 m if laid end to end. The filtrate is very dilute and is mostly water. Of some 200 L of filtrate a day, an average adult concentrates about 1.5 L of urine. It is obviously essential that most of the filtrate and many of its dissolved materials be reabsorbed, while only harmful materials are excreted. This is a function of the kidney tubules (Figure 7.4), in which residues are gradually concentrated into urine.

Generally, the filtration of macromolecules through the glomerular capillary wall is inversely proportional to the molecular weight of a substance: Small molecules are freely filtered, while large molecules, such as certain proteins, are restricted. Filtration of anionic molecules is more restricted than neutral or cationic molecules of the same size. Toxicants that neutralize or decrease the number of fixed anionic charges on glomerular structural elements will impair the charge- or size-selective properties of the glomerulus, leading to urinary excretion of polyanionic or high molecular weight proteins (Schnellmann 2001).

Environmental chemicals, including metals and drugs, may be transported across proximal tubular cells (i.e., from renal capillaries across tubular cells to be excreted in tubular lumen or vice versa). Many cationic substances are excreted against concentration gradients at rates greater than the glomerular filtration rate. This indicates an active transport process. Such a process requires expenditure of energy derived from oxidative metabolism carried out in mitochondria. However, active transport with the capability of concentrating absorbed material may concentrate potential nephrotoxins as well as essential substances in the renal cortex. The same toxins that cause adverse effects on energy metabolism will impede the cellular transport of essential solutes. Other toxic substances may also be concentrated in the medulla.

As noted previously, metabolism of chemicals within the kidney may result in substances that are either more or less toxic than the parent chemicals. For instance,

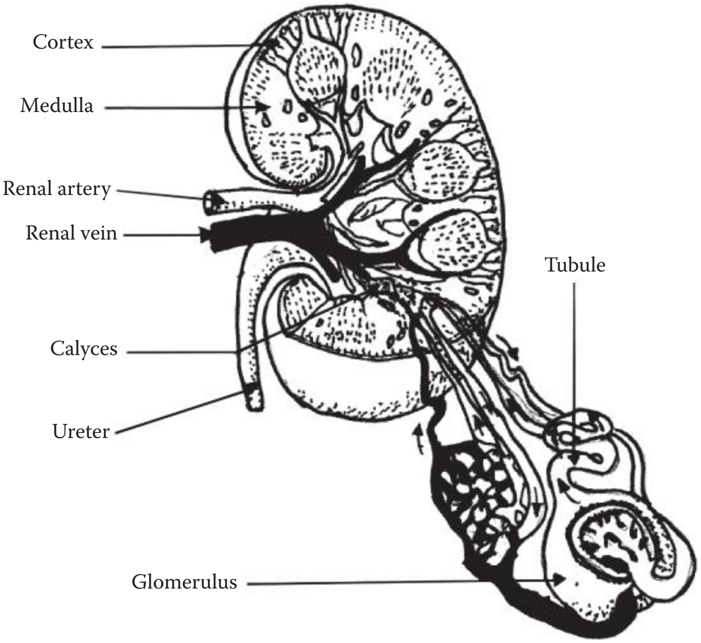

FIGURE 7.4 Structure of human kidney.

$CHCl_3$ and CCl_4 may be biotransformed into reactive, toxic products (Chapter 4) that bind covalently to renal tissue, leading to membrane injury. Exposure to certain other substances may result in activation or enhancement of enzyme systems such as the mixed-function oxidase (MFO). The toxicity of methoxyfluorane, for example, may be enhanced as a result of increased metabolism as the metabolic products (i.e., fluoride and oxalate) are both known to be potentially toxic to the kidney. Fluoride ions are toxic to cell membranes, whereas oxalate may accumulate within the lumen of nephrons.

Heavy metals such as lead, cadmium, and mercury are known also to cause renal disease. The adverse effects of lead may be both acute and chronic. Cells of the proximal tubules are most severely affected, as shown by reduction in resorptive function of nutrients such as glucose and amino acids. On the other hand, the effect of inorganic cadmium salts on the kidney is largely chronic. The characteristics of cadmium nephropathy include increased cadmium in the urine, proteinuria, aminoaciduria, glucosuria, and decreased renal reabsorption of phosphate. With chronic exposure to toxic levels, renal tubular acidosis, hypercalciuria, and calculi formation occur (Goyer 1985). Mercury is known to produce different effects on kidneys, depending on the biochemical form of the metal and nature of exposure. Inorganic mercury compounds can cause acute tubular necrosis, whereas chronic low-dose exposure to mercuric salts or elemental mercury vapor may induce an immunologic glomerular disease. The presence of proteins rich in cysteine may be able to alleviate mercury toxicity. As noted, selenium is known to antagonize mercury, reducing its toxicity.

An interesting phenomenon concerning the toxicity of cadmium is the role that metallothionein (MT) plays. MTs are low molecular weight, nonenzymatic proteins and are ubiquitous in the animal kingdom. They have unique amino acid composition as they do not contain aromatic amino acids but are rich in cysteine (which consists of one-third of the amino acid residues), and thus are capable of binding metals such as zinc and cadmium. Various physiologic stimuli can induce MT genes. The formation of MTs following exposure to cadmium appears to protect the body against its toxicity (Klassen et al. 1999).

The mammalian kidney is unusually susceptible to the toxic effects of various noxious chemicals. This is attributed partly to the unique physiologic and anatomical features of the kidney. Although the kidneys receive 20% to 25% of the resting cardiac output, they only make up about 0.5% of the total body mass. Thus, relatively high amounts of any chemical or drug in the systemic circulation will be delivered to the kidneys. Since kidneys are the organs involved in forming concentrated urine, they tend to concentrate potential toxicants in the tubular fluid as well. Therefore, a nontoxic level of toxicants in the plasma may reach toxic levels in the kidney. Moreover, as noted, kidneys are involved in renal transport, accumulation, and metabolism of xenobiotics. As kidneys participate in these processes, they will clearly increase their susceptibility to toxic injury (Schnellmann 2001).

REVIEW QUESTIONS

1. Briefly explain what metallothionein (MT) is.
2. What is unique about the amino acid composition of MT?
3. What are phytochelatins?
4. What is the function of phytochelatins?
5. What are the compositional characteristics of phytochelatins?
6. What is chronic bronchitis?
7. What is the function of alveoli?
8. Which of the following types of cells are responsible for the exchange of O_2 and CO_2? (a) trachea; (b) bronchi; (c) alveoli; (d) respiratory capillary
9. What is emphysema? Briefly explain how it may occur.
10. What is the function of a macrophage? And how does it perform its function?
11. Which environmental agents can affect the function of macrophages?
12. What is the composition of the membranes of mammalian cells?
13. Explain the characteristics of the phospholipid bilayer in membranes.
14. What is the reason for the kidneys to be susceptible to toxic injury?
15. Explain how heavy metals such as lead and cadmium may damage membranes.
16. What substances in plant tissues may cope with cellular free radicals?
17. What enzymes in plant cells can inhibit the action of oxidants? Explain how they perform such functions.

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8 Air Pollution

Inorganic Gases

8.1 INTRODUCTION

This chapter discusses four of the major gaseous air pollutants: sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3) (ground-level), and carbon monoxide (CO). The importance of these gaseous air pollutants is clear from the fact that they are four of the six "Criteria Air Pollutants" regulated by the U.S. Environmental Protection Agency (EPA). The other two criteria air pollutants are particulate matter and lead (Pb), which are discussed in Chapter 9.

8.2 SULFUR DIOXIDE

SO_2 and sulfur trioxide (SO_3) are the two sulfur oxides (SO_x) that are important air pollutants. This chapter focuses on SO_2 because it is far more important than SO_3 as an air pollutant. In fact, based on the quantities emitted into the atmosphere, SO_2 is considered the most damaging gaseous pollutant.

8.2.1 SOURCES OF SO_2

Atmospheric SO_2 arises from both natural and anthropogenic sources. Sulfur (S) compounds are emitted naturally through volcanic actions, sea salt over the oceans, and decomposition of organic matter (mostly as hydrogen sulfide, H_2S). About 95% of the anthropogenic emissions of sulfur to the atmosphere are in the form of SO_2. The main human activities that cause its emission include combustion of coal and petroleum products, petroleum refining, and nonferrous smelting. In the United States, about 95% of the total SO_2 emission is from industry and stationary sources.

The sulfur content of coal ranges from 0.3% to 7%, and it is present in both organic and inorganic forms. In oil, the sulfur content ranges from 0.2% to 1.7%, and it is in organic form. The most important sulfur-containing compound in coal is iron disulfide or pyrite (FeS_2). When heated to high temperatures, pyrite is oxidized to $FeSO_4$ or Fe_2O_3 and SO_2 through the following reactions:

$$FeS_2 + 3O_2 \rightarrow FeSO_4 + SO_2 \tag{8.1}$$

$$4FeS_2 + 11O_2 \rightarrow 2Fe_2O_3 + 8SO_2 \tag{8.2}$$

In the smelting process, sulfide ores of copper (Cu), lead, and zinc (Zn) are oxidized (roasted), forming metallic oxides. For example, zinc sulfide (ZnS) is converted in a smelter to zinc oxide (ZnO), releasing SO_2:

$$2ZnS + 3O_2 \rightarrow 2ZnO + 2SO_2 \tag{8.3}$$

8.2.2 CHARACTERISTICS OF SO_2

SO_2 is highly soluble in water, with a solubility of 11.3 g/100 mL. Once emitted into the atmosphere, it can dissolve in fog or cloud droplets, forming sulfurous acid (H_2SO_3), which is readily oxidized by molecular oxygen (O_2) to sulfuric acid (H_2SO_4). The formation of H_2SO_4 by this process is greatly facilitated by some metal salts, which are also dissolved in the droplets. Any ammonia (NH_3) present in the atmosphere will rapidly react with H_2SO_3 or H_2SO_4 droplets to form ammonium sulfate $(NH_4)_2SO_4$ or ammonium bisulfate $(NH_4)HSO_4$ (Kellogg et al. 1972).

Atmospheric SO_2 may be removed by several competing processes: direct removal by deposition as bisulfate in precipitation; incorporation into fog and cloud droplets (where it is oxidized catalytically and photochemically to sulfate); or diffusion to plant surfaces, where it is adsorbed and reacts chemically. Gaseous SO_2 may also be dissolved in water droplets and oxidized, forming H_2SO_4 aerosol droplets. According to Fox (1986), both dry and wet forms of H_2SO_4 produced in the atmosphere may be removed by deposition to the earth's surface (Figure 8.1).

8.2.3 EFFECTS ON PLANTS

SO_2 enters plant leaves predominantly by gaseous diffusion through stomatal pores, as do other atmospheric pollutants. The number of stomata and the size of aperture are important factors affecting SO_2 uptake. Other factors, such as light, humidity, temperature, and wind velocity, are also important because they influence the turgidity of guard cells. At low concentrations, SO_2 can injure epidermal and guard cells, inducing elevated stomatal conductance and greater entry of the gas into plant tissues.

Following uptake by plant leaves, SO_2 is rapidly translocated through the plant. It can then affect photosynthesis, transpiration, and respiration—the three major functions of plant leaves. A slight increase in both net photosynthesis and transpiration may occur at low SO_2 concentrations for short periods, followed by a decrease in both processes. At higher SO_2 concentrations, both these processes decrease. Plant injuries may be manifested by leaf chlorosis and spotty necrotic lesions (Figure 8.3). As noted previously, a synergistic effect on leaf damage occurs when plants are exposed to SO_2 and O_3 simultaneously. Damage to mesophyll cells commonly occurs, which is the main cause of observed changes in photosynthesis. Exposure of Chinese guger-tree seedlings grown in field chambers to 325 ppb of SO_2 for 4 weeks showed rapid decreases in photosynthetic rate, root weight, and total seedling weight (Sheu 1994). A simultaneous increase (75%) in –SH groups in leaves also occurred.

Once absorbed into a leaf, SO_2 readily dissolves in the intercellular water to form bisulfite (HSO_3^-), sulfite (SO_3^{2-}), and other ionic species (Figure 8.4). Both HSO_3^- and SO_3^{2-} have a lone pair of electrons on the sulfur atom that strongly favors reactions

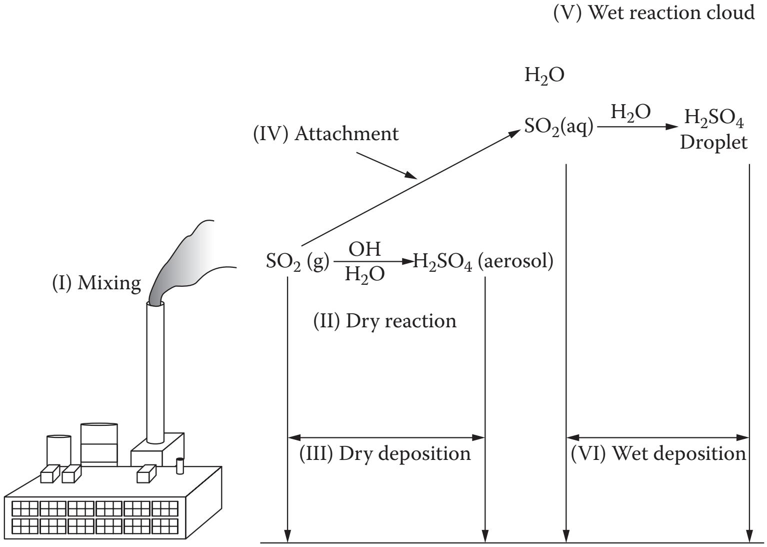


FIGURE 8.1 SO_2 transport, transformation, and deposition processes. Initially, SO_2 is mixed into the atmosphere (I). Gaseous SO_2 may undergo oxidation in the gaseous phase with subsequent formation of H_2SO_4 aerosol (II). Both gaseous SO_2 and H_2SO_4 aerosol may be deposited at the earth's surface (III). Gaseous SO_2 may become dissolved in a water droplet (IV). The dissolved SO_2 can be oxidized in solution to form H_2SO_4 aerosol droplets (V). The H_2SO_4 aerosol and the H_2SO_4 droplets may be removed to the earth's surface by wet deposition (VI). (Adapted and redrawn from Fox, D.L. In *Air pollution*, 3rd ed., Vol. 6, A.C. Stern, Ed. Academic Press, New York, 1986, 86–87.)

with electron-deficient sites in other molecules. They are both phytotoxic and can affect several physiological and biochemical processes in plants (Plesnicar 1983). The phytotoxicity of SO_3^{2-} and HSO_3^- is diminished when these ions are converted to less-toxic forms, such as sulfate (SO_4^{2-}). For instance, oxidation of HSO_3^- to SO_4^{2-} can occur both enzymatically and nonenzymatically. Several factors, including cellular enzymes such as peroxidase and cytochrome oxidase, metals, UV (ultraviolet) light, and O_3, stimulate the oxidation of SO_2. In the presence of SO_3^{2-} and HSO_3^-, more O_2 is formed by free-radical chain oxidation. Other free radicals may also be formed. These oxidizing radicals can have detrimental effects on leaf cells. On the other hand, SO_3^{2-} and SO_4^{2-} formed may be reduced and assimilated with a carbon skeleton to cysteine (an amino acid) (Carsed 1985).

Plant metabolism is affected by SO_2 in a variety of ways: stimulation of phosphorus metabolism and reduction in foliar chlorophyll concentration (Lauenroth and Dodd 1981), increase or decrease in carbohydrate concentrations in red kidney bean plants exposed to low or high levels of SO_2 (Koziol and Jordon 1978), and inhibition of lipid biosynthesis in pine needles treated with SO_2 (Malhotra and Khan 1978). According to Malhotra and Khan (1978), pine needle tissues, particularly the developing tissues, actively incorporate acetate [1–^{14}C] into phosphogalacto- and neutral lipids. The major incorporation of the label among these lipids was always in the phosphatidyl choline fraction. Treatment of needle tissues with gaseous or

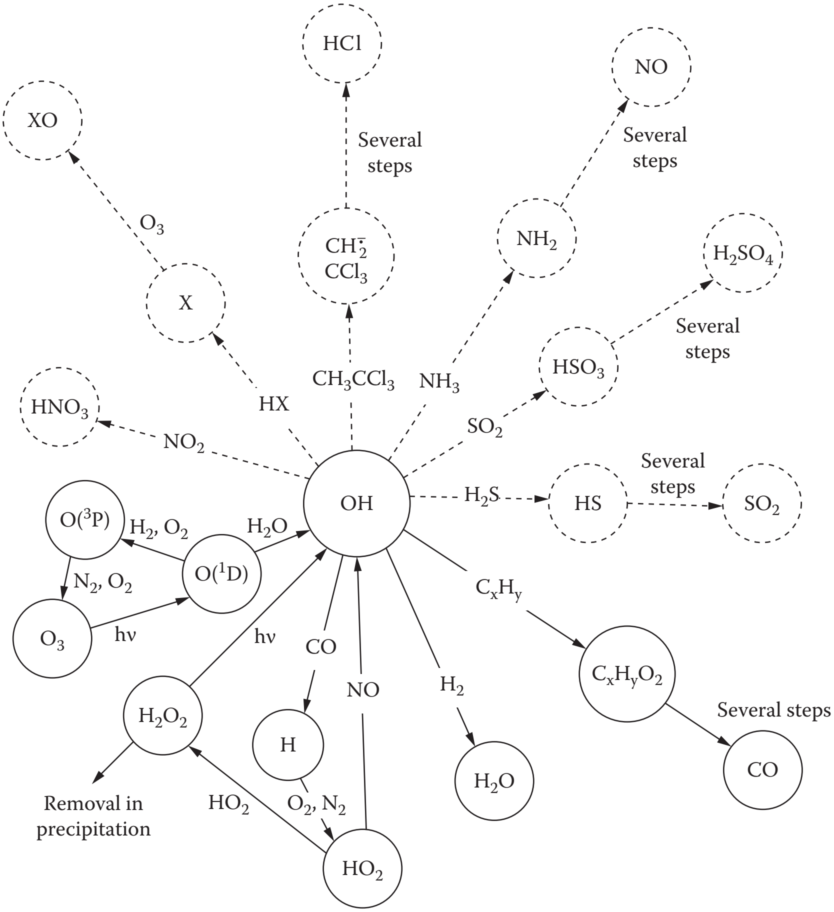

HCl
XO
NO
Several steps
Several steps
O_3
CH_2
CCl_3
NH_2
H_2SO_4
X
Several steps
HSO_3
HNO_3
HX
CH_3CCl_3
NH_3
SO_2
NO_2
Several steps
OH
H_2S
HS
SO_2
$O(^3P)$
H_2, O_2
H_2O
$O(^1D)$
N_2, O_2
C_xH_y
O_3
hν
hν
CO
NO
H_2
$C_xH_yO_2$
H_2O_2
H
Several steps
H_2O
CO
Removal in precipitation
HO_2
O_2, N_2
HO_2

FIGURE 8.2 Photochemistry of OH· radical controls trace gas concentration. The photochemistry of the free hydroxyl radical controls the rate at which many trace gases are oxidized and removed from the atmosphere. Processes that are of primary importance in controlling the concentration of OH· in the troposphere are indicated in a solid line in the schematic diagram; those that have a negligible effect on OH· levels but are important because they control the concentrations of associated reactions and products are indicated in a broken line. Circles indicate reservoirs of species in the atmosphere; arrows indicate reactions that convert one species to another, with the reactant or photon needed for each reaction indicated along each arrow. Multistep reactions actually consist of two or more sequential elementary reactions. HX = HC1, HBr, HI, or HF. C_xH_y denotes hydrocarbons. (Adapted from Chameides, W.L., and D.D. Davis. Chemistry in the troposphere. *C&EN,* 1982. With permission from American Chemical Society.)

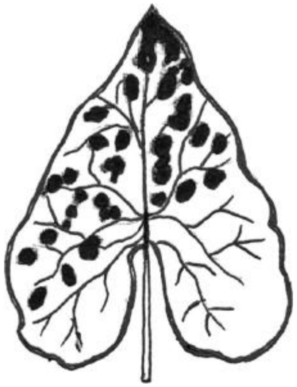


FIGURE 8.3 Leaf necrosis (spotty lesions) induced by SO_2.

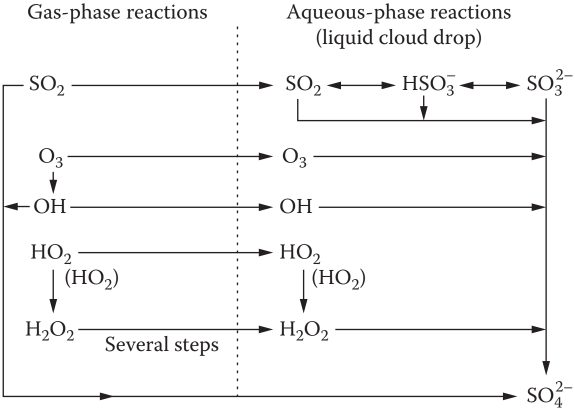

Gas-phase reactions
Aqueous-phase reactions (liquid cloud drop)
SO_2
SO_2
HSO_3^-
SO_3^{2-}
O_3
O_3
OH
OH
HO_2
(HO_2)
HO_2
(HO_2)
H_2O_2
Several steps
H_2O_2
SO_4^{2-}

FIGURE 8.4 Fate of SO_2 present in tissues. Note: Arrows crossing liquid cloud drop barrier signify heterogeneous reactions that transfer a species from the gas phase to the aqueous phase. (Adapted from Chameides, W.L., and D.D. Davis. Chemistry in the troposphere. *C&EN,* 1982. With permission from American Chemical Society.)

aqueous SO_2 markedly inhibited lipid biosynthesis. A partial or complete recovery in lipid biosynthesis capacity in plants occurred when they were removed from the SO_2 environment.

SO_2 affects a number of enzymes in different plant species. Examples include alanine and aspartate aminotransferases, glutamate dehydrogenase, malate dehydrogenase, glycolate oxidase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, fructose-1,6-bisphosphatase, ribulose-5-phosphate kinase, peroxidase, and SOD. Enzyme activity may be enhanced or inhibited by exposure to SO_2 at different concentrations. With Chinese Guger-tree seedlings exposed to 325 ppb of SO_2, for example, their peroxidase activity increased significantly, while SOD activity was unaffected (Sheu 1994).

The tolerance of plants to SO_2 depends on plant species under similar biophysical conditions. This suggests that delicate biochemical and physiological differences in plants could affect the sensitivity of a particular plant species to SO_2.

8.2.4 Effects on Animals

Although SO_2 is an irritating gas for the eyes and upper respiratory tract, no major injury from exposure to any reasonable concentrations of the gas in the atmosphere has been demonstrated in experimental animals. Even exposure to pure gaseous SO_2 at concentrations 50 or more times ambient values produced little distress (Alarie et al. 1970, 1973). Concentrations 100 or more times the ambient value are required to kill small animals. Mortality is associated with lung congestion and hemorrhage, pulmonary edema, thickening of the interalveolar septa, and other relatively nonspecific changes of the lungs, such as pulmonary hemorrhage and hyperinflation. These changes were associated with salivation, lacrimation, and rapid shallow ventilation. In mice exposed to 10 ppm SO_2 for 72 hours, necrosis and sloughing of the nasal epithelium were observed (Giddens and Fairchild 1972). The lesions were more severe in animals with preexisting infection. Other symptoms include decreased weight gains, loss of hair, nephrosis in kidneys, myocardial degeneration, and accelerated aging.

The health effects of acidic aerosols on experimental animals have been studied extensively. Changes in pulmonary function, particularly increases in pulmonary flow resistance, occur after acute exposure. H_2SO_4 is shown to be more irritating than any of the sulfate salts in this regard. The irritant effect of the acid depends in part on the size of the droplets, with smaller droplets being more irritating (Amdur et al. 1978). Animals exposed to 0.3 to 0.6 μm H_2SO_4 droplets at various concentrations, for example, showed either slowed or accelerated bronchial mucociliary clearance function depending on the concentration of the aerosol. Studies on the comparative effects of exposure to H_2SO_4 and ammonium bisulfate (NH_4HSO_4) showed alteration of phagocytic activity, with more pronounced effect exhibited by H_2SO_4. Repeated exposures to H_2SO_4 caused the production of hyperresponsive airways in previously healthy animals. Such exposure also resulted in histological changes, such as increased numbers of secretory cells in distal airways and thickened epithelium in airways of midsize bronchi and terminal bronchioles (Schlesinger et al. 1983).

8.2.5 Health Effects

Epidemiological evidence from studies during the London episodes (Chapter 3) suggests that effects of SO_2 may be manifested at or above 0.19 ppm (24-hour average) in combination with elevated particle levels. Short-term, reversible declines in lung function at SO_2 levels above 0.10 to 0.18 ppm may occur. These effects may be caused by SO_2 alone or by H_2SO_4 or other irritant aerosols. The role of SO_2 appears more likely to involve transformation products such as acidic fine particles. H_2SO_4 and sulfates have been shown to influence both sensory and respiratory function, such as increased respiratory rates and tidal volumes, and slowing of mucus clearance in humans (Horstman et al. 1988).

The effect of SO_2 on human health varies markedly with the health status and physical activity of the individuals. For example, in asthmatics and others with hyperreactive airways exposed to SO_2 at 0.25 to 0.50 ppm and higher while exercising, the most striking response was rapid bronchoconstriction (airway narrowing). This is usually demonstrated by elevated airway resistance, lowered expiratory flow rates, and the manifestation of symptoms such as wheezing and shortness of breath. The time required for significant bronchoconstriction to occur in exercising asthmatics is brief. Exposure durations as short as 2 minutes at 1.0 ppm have produced significant responses (Horstman et al. 1988). The combined effect of SO_2 and cold, dry air exacerbates the asthmatic response (Sheppard et al. 1984). The bronchoconstrictive effects of SO_2 are reduced under warm, humid conditions (Linn et al. 1985).

Exposure to submicrometer-size H_2SO_4 aerosols increases tracheobronchial and alveolar rates of clearance in humans. Although the altered clearance rates may be an adaptive response of the mucociliary system to acid exposures, they may also be early stages in the progression toward more serious dysfunctions, such as chronic bronchitis. Many researchers consider that chronic bronchitis in all exposed persons may result from continued irritant exposures. In asthmatics, inhalation of acidic aerosols may lead to bronchospasm.

Certain morphological changes are associated with the observed clinical symptoms in human chronic bronchitis. The changes include an increase in the number or size of epithelial mucous secretory cells in both proximal bronchi and peripheral airways. The changes are accompanied by an increase in the volume of secretion (Reid 1963). These changes are followed by an increase in epithelial thickness and a decrease in airway diameter, similar to those observed in experimental animals.

Synergism may be observed in elevated airway resistance induced by SO_2 in combination with certain other air pollutants. For example, the response to inhaled SO_2 can be exacerbated by prior exposure to O_3. Also, the presence of H_2SO_4 on ultrafine ZnO particles (simulating coal combustion effluent) in a mixture with SO_2 has been shown to increase lung reactivity responses by 10-fold over those produced by pure droplets of H_2SO_4 of comparable size (Amdur and Chen 1989).

Published reports supported the hypothesis that acidic pollutants contribute to carcinogenesis in humans. Researchers have also examined possible biological mechanisms for such contribution. They include pH modulation of toxicity of xenobiotics and pH-dependent alteration of cells involving mitotic and enzyme regulation. Based on review of the mortality data from London in the period 1958 to 1972, the EPA (1986) concluded that marked increases in mortality occurred, mainly among the elderly and chronically ill, and that the increases were associated with black smoke and SO_2 concentrations above 1,000 $\mu g/m^3$. The conclusion was especially favored when such an elevation of pollutants occurred for several consecutive days.

It is widely known that China's steady increase in coal burning in recent decades has resulted in an explosive increase in sulfur pollution. Based on studies of some scientists at the National Oceanic and Atmospheric Administration in Boulder, Colorado, Chinese coal burning appears to have contributed to a steady increase since 2000 of the sulfurous haze 20 to 30 km above ground levels. According to the scientists, the global annual sulfur emissions of 50 million tons or so had not made a noticeable health impact until China's economy took off, increasing its sulfur emissions more than 60% between 2000 and 2005. They pointed out that these increased emissions would account for the observed 4% to 7% per year thickening of stratospheric haze (*Science* 2009).

8.3 NITROGEN DIOXIDE

8.3.1 Forms and Formation of Nitrogen Oxides

There are six forms of nitrogen oxides (NO_x) that occur in the atmosphere: nitrous oxide (N_2O), nitric oxide (NO), nitrogen dioxide (NO_2), nitrogen trioxide (N_2O_3), nitrogen tetroxide (N_2O_4), and nitrogen pentoxide (N_2O_5). Of these, NO_2 is the most important air pollutant because of its relatively high toxicity and its ubiquity in ambient air, while N_2O, N_2O_3, and N_2O_4 have been considered to have relatively low toxicity and significance as air pollutants. However, recent studies showed that N_2O is an extremely important gas contributing to ozone depletion in the stratosphere (see Section 8.3.6).

Basic chemical reactions involving NO_2 formation are as follows:

$$N_2 + O_2 \xrightarrow{1{,}210^\circ C} 2NO \tag{8.4}$$

$$2NO + O_2 \rightarrow 2NO_2 \tag{8.5}$$

The NO formed in Equation 8.4 persists when the temperature is cooled rapidly, as is the case in ambient air. The reaction shown in Equation 8.5 is one of the few reactions that are slowed with an increase in temperature.

8.3.2 Major Reactive Nitrogen Species in the Troposphere

Several reactive nitrogen species, including NO, NO_2, and HNO_3, occur in the troposphere. Among these, NO_2 is of particular environmental concern because it plays a complex and important role in the production of photochemical oxidants and acidic deposition. NO_2 is a unique air pollutant because it absorbs UV light energy and is then broken down to NO and atomic oxygen. The energetic oxygen atom reacts with molecular oxygen to form O_3. The resultant O_3 then reacts with NO to form molecular oxygen and NO_2, thus terminating the photolytic cycle of NO_2 (Figure 8.5). It is clear from the equation that, as far as the cycle is concerned, there is no net gain or loss of chemical substances. However, accumulation of O_3 does occur, for reasons that are discussed in the following section. With numerous photochemical reactions occurring in the troposphere, production of photochemical smog ensues.

In addition to NO and NO_2, nitric acid (HNO_3) is also an important nitrogen compound in the troposphere. Although HNO_3 is produced mainly from the reaction between NO_2 and OH·, it is formed through a secondary reactive pathway as well. In this case, NO_2 is first oxidized to NO_3 by O_3. The resultant NO_3 reacts with a molecule of NO_2, producing N_2O_5. The N_2O_5 combines with a molecule of water, yielding HNO_3. HNO_3, in turn, may be precipitated through rainout or dry deposition (Figure 8.6).

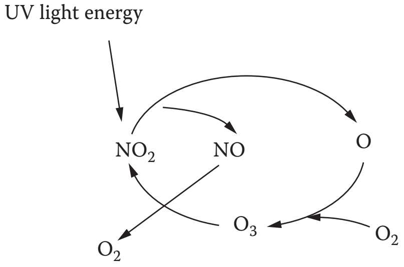

UV light energy
NO_2
NO
O
O_3
O_2
O_2

FIGURE 8.5 The photolytic cycle of NO_2.

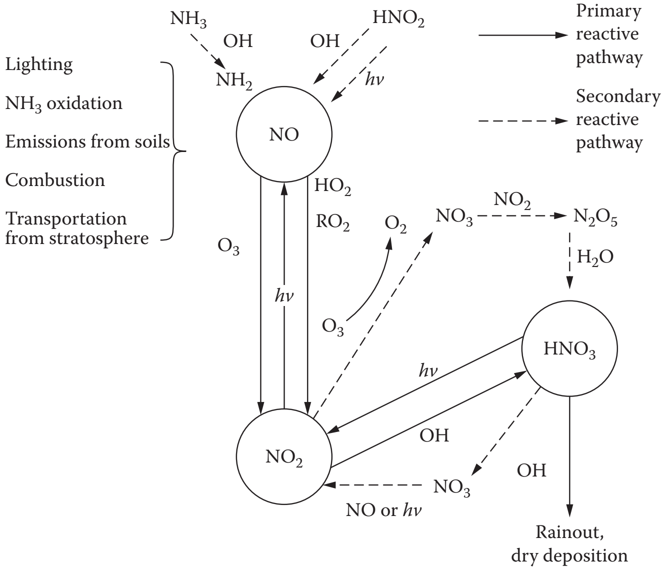

NH_3
OH
NH_2
HNO_2
OH
hv
Lighting
NH_3 oxidation
Emissions from soils
Combustion
Transportation from stratosphere
NO
O_3
hv
HO_2
RO_2
O_2
O_3
NO_3
NO_2
N_2O_5
H_2O
HNO_3
hv
OH
NO_2
NO_3
NO or hv
OH
Rainout, dry deposition
Primary reactive pathway
Secondary reactive pathway

FIGURE 8.6 Major reactive nitrogen species in troposphere. (Adapted from Chameides, W.L., and D.D. Davis. Chemistry in the troposphere. *C&EN*, 1982. With permission from American Chemical Society.)

8.3.3 EFFECTS ON PLANTS

Plant absorbs gaseous NO_x through stomata. NO_2 is more rapidly absorbed than NO, mainly because of its rapid reaction with water. NO is almost insoluble in an aqueous medium. The absorbed NO_2 is converted to nitrate (NO_3^-) and nitrite (NO_2^-) ions before the plant can metabolize it. NO_2-induced plant injury may be due to either acidification or a photooxidation process (Zeevaart 1976). Symptoms exhibited by plants exposed to NO_2 are similar to those observed in plants exposed to SO_2, but much higher concentrations are required to cause acute injury. However, decreased photosynthesis has been demonstrated even at concentrations that do not produce visible injury. The combined effect of NO and NO_2 gases appears to be additive.

Photosynthetic inhibition caused by NO_x may be due to competition for NADPH (nicotinamide adenine dinucleotide phosphate) between the processes of nitrite reduction and carbon assimilation in chloroplasts. NO_2 has been shown to cause swelling of chloroplast membranes (Zeevaart 1976). Biochemical and membrane injuries may be caused by NH_3 produced from NO_3^- if NH_3 is not utilized soon after its formation. Plants can metabolize the dissolved NO_x through their NO_2 assimilation pathway as shown below:

$$NO_x \rightarrow NO_3^- \rightarrow NO_2^- \rightarrow NH_3 \rightarrow \text{Amino acids} \rightarrow \text{Proteins}$$

Other biochemical pathways affected by NO_x include inhibition of lipid biosynthesis, oxidation of unsaturated fatty acids in vivo, and stimulation of peroxidase activity.

8.3.4 Health Effects

Studies of the pathological and physiological effects of NO_2 on animals have been conducted at concentrations much higher than those found in ambient air. The toxic action of NO_2 is mainly on the deep lung and peripheral airway. In various species of animals studied, exposure to NO_2 at 10 to 25 ppm for 24 hours was shown to induce the formation of fibrin in the airway, an increased number of macrophages, and altered appearance of the cells in the distal airway and adjacent pulmonary alveoli. Terminal bronchioles showed hyperplasia and hypertrophy, loss of cilia, and disturbed ciliagenesis. Large crystalloid depositions also occurred in the cuboidal cells. Continuous exposure for several months produced thickening of the basement membranes, resulting in narrowing and fibrosis of the bronchioles. Emphysema-like alterations of the lungs developed, followed by death of the animals (Wellburn 1972).

As mentioned, although almost all the studies reported were conducted using much higher concentrations of NO_2 than are found in ambient air, a few studies have dealt with low NO_2 concentrations. Orehek et al. (1976) showed that asthmatic subjects exposed to 0.1 ppm of NO_2 developed significantly aggravated hyperreactivity in the airway. While the health effects of prevailing concentrations of NO_2 are generally considered insignificant, NO_2 pollution may be an important aspect of indoor pollution. Evidence suggests that gas cooking and heating of homes, when not well vented, can increase NO_2 exposure, and that such exposure may cause increased respiratory problems among individuals, particularly young children.

NO_2 is highly reactive and has been reported to cause bronchitis and pneumonia, as well as to increase susceptibility to respiratory infections (Table 8.1) (Romieu 1999) Epidemiological studies suggested that children exposed to NO_2 are at a higher risk of respiratory illness. NO_2 exposure has been shown to impair immune responses and has been associated with daily mortality in children less than 5 years old, as well as increased intrauterine mortality levels in São Paulo, Brazil (Pereira et al. 1998).

8.3.5 Biological Effects

Inhaled NO_2 is rapidly converted to NO_2^- and NO_3^- ions in the lungs, and these ions will be found in the blood and urine shortly after exposure to 24 ppm of NO_2 (Freeman

TABLE 8.1
Health Effects Associated with NO_2 Exposure in Epidemiological Studies

Health Effect	Mechanism
Increased incidence and severity of respiratory infections	Reduced efficacy of lung defenses
Reduced lung function	Airway and alveolar injuries
Respiratory symptom	Airway injury
Worsening clinical status of persons with asthma, chronic obstructive pulmonary disease, or other chronic respiratory conditions	Airway injury

Source: Adapted from Romieu, I. In *Urban traffic pollution.* Ecotox/WHO/E&FN Spon, London, 1999, 9.

and Haydon 1964). Increased respiration was shown in some studies. Other physiological alterations include a slowing of weight gain and decreased swimming ability in rats, alteration in blood cellular constituents such as polycythemia, lowered hemoglobin content, thinner erythrocytes, leukocytosis (an increase in the number of leukocytes in the circulating blood), and depressed phagocytic activity. Methemoglobin formation occurred only at high concentrations. Methemoglobinemia is a disorder manifested by high concentrations of methemoglobin in the blood. Under this condition, the hemoglobin contains an Fe^{3+} ion and is thus unable to reversibly combine with molecular oxygen. The lipid material extracted from the lung of rats exposed to NO_2 has revealed that oxidation had occurred. Also, lipid peroxidation was more severe in animals fed a diet deficient in vitamin E (Pereira et al. 1998). In contrast to O_3, reaction of NO_2 with fatty acids appears to be incomplete, and phenolic antioxidants can retard the oxidation from NO_2.

Exposure to NO_2 may cause changes in the molecular structure of lung collagen. In a series of studies, Buckley and Balchum (1967a) showed that exposure to 10 ppm NO_2 for 10 weeks or longer or exposure to 50 ppm NO_2 for 2 hours increased both tissue oxygen consumption and the activities of lactate dehydrogenase and aldolase. Stimulation of glycolysis has also been reported.

8.3.6 N_2O AND STRATOSPHERIC O_3 LAYER DEPLETION

Concerns about the stratospheric ozone layer have largely focused on reactions of O_3 with Cl^- and Br^- atoms released from the atmospheric dissociation of chlorofluorocarbons and other anthropogenic halocarbons. Meanwhile, concerns about human-induced effects on global climate have concentrated on CO_2 and CH_4 emissions from fossil fuels and other sources. However, rising atmospheric concentrations of nitrous oxide (N_2O) are contributing to global warming and stratospheric ozone destruction (NAS/NRC 1977). Studies by Ravishankara et al. (2009) have shown that future changes in climate and in the destruction of stratospheric O_3 depend on the emissions and changing atmospheric concentration of N_2O. Their report not only adds to the scientific understanding of this important gas but also is a strong reminder that N_2O deserves much more attention. According to their studies, the atmospheric N_2O concentration has been increasing at roughly 0.25% per year, and this trend is set to continue. N_2O is mainly removed from the atmosphere through photolysis and reaction with excited oxygen atoms in the middle to upper stratosphere, resulting in a long atmospheric lifetime, which is roughly 120 years.

About 70% of atmospheric N_2O emissions are natural, mostly from bacterial breakdown of N_2 in soils and in the oceans. Human activities are responsible for the remaining 30% of N_2O emissions. The largest human-related source of the gas comes from agricultural practices and activities, including the use of synthetic and organic fertilizers, production of nitrogen-fixing crops, and application of livestock manure to croplands and pasture. N_2O can also be produced during fossil fuel combustion. The vast majority of the reactive NO_x in the stratosphere results from the dissociation of N_2O (through its reaction with electronically excited oxygen atoms). As a result of the high reactivity of NO_x with O_3, N_2O levels in the preindustrial atmosphere were sufficient to account for the majority of the natural destruction of O_3 in the atmosphere. With increasing atmospheric concentration of N_2O, the concentrations of NO_x in the stratosphere are also rising. Ravishankara et al. (2009) have shown that NO_x and, as a result, N_2O destroy more O_3 in the current stratosphere than does any other reactive chemical family.

The execution of the Montreal Protocol effectively controls the emission of some major O_3-depletion gases, particularly gases containing Cl^- and Br^-. If one assumes that only halocarbons from human activities affect ozone and that there is full global compliance with the Montreal Protocol, the O_3 layer outside the polar region is largely expected to recover from existing human effects on the stratosphere by the middle of the twenty-first century.

The increasing concentration of CO_2 in Earth's atmosphere not only warms the troposphere but also cools the stratosphere, with a tendency to increase the amount of stratospheric O_3. As a result, there is a possibility of a "superrecovery," in which the total amount of atmospheric O_3 will exceed that found before 1980, before the major O_3 losses due to halocarbons occurred. Furthermore, changes in climate are altering the strength of circulation patterns in the stratosphere, thus affecting the distribution of O_3. Potentially even more important is the continuing increase in atmospheric concentrations of N_2O and CH_4. Methane affects the amount of hydrogen oxides in both the troposphere and stratosphere, which in turn affects the chemistry of O_3. Whereas increasing N_2O will tend to destroy more O_3, increasing CH_4 would tend to produce O_3, but each has its largest effects at different locations in the stratosphere (Figure 8.7).

In addition to its effects on O_3, N_2O is the third-most-important gas directly affecting climate as a result of human activities. Although the increases in concentrations

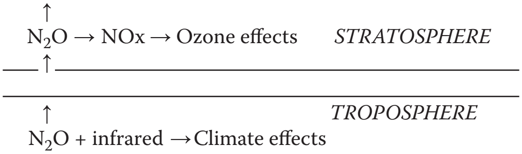

N_2O → NOx → Ozone effects
STRATOSPHERE
TROPOSPHERE
N_2O + infrared → Climate effects

FIGURE 8.7 Effects of N_2O on stratospheric ozone and climate.

of CO_2 and CH_4 have been larger, N_2O is also a greenhouse gas, and its changing concentrations are important to climate change. However, even if the combustion-related sources prove to be relatively easy to control, the agricultural sources may present a large challenge. Greater demand for food may affect the ability to reduce emissions from livestock and the use of fertilizers.

By comparing the weighted ozone depletion potential (ODP) of anthropogenic emissions of N_2O with those of other O_3-depleting substances, NO_2 emission is expected to remain the largest throughout the twenty-first century. N_2O is unregulated by the Montreal Protocol. It is suggested that limiting future N_2O emissions would enhance the recovery of the O_3 layer from its depleted state and would reduce the anthropogenic forcing of the climate system.

The depletion of the stratospheric O_3 layer by human-made chemicals, referred to as ozone-depleting substances (ODSs), was one of the major environmental issues of the twentieth century. The Montreal Protocol on Substances that Deplete the Ozone has been highly successful in reducing the emissions, growth rates, and concentrations of chlorine- and bromine-containing halocarbons, the historically dominant ODSs, and has limited O_3 depletion and initiated the recovery of the O_3 layer.

Nitrogen oxides ($NO_x = NO + NO_2$) are also known to catalytically destroy O_3 via the following reactions:

$$NO + O_3 \rightarrow NO_2 + O_2 \tag{8.6}$$

$$O + NO_2 \rightarrow NO + O_2 \tag{8.7}$$

$$\text{Net: } O + O_3 \rightarrow 2O_2 \tag{8.8}$$

The primary source of stratospheric NO_2 is surface N_2O emissions. N_2O has been thought of as primarily a natural atmospheric constituent, but the influence of its changes on long-term changes in O_3 concentrations has recently been investigated.

N_2O shares many similarities with the chlorofluorocarbons (CFCs), historically the dominant ODSs. The CFCs and N_2O are very stable in the troposphere, where they are emitted, and are transported in the stratosphere, where they release active chemicals that destroy stratospheric O_3 through chlorine- or nitrogen oxide-catalyzed processes. They both have substantial anthropogenic sources. Unlike CFC, N_2O also has natural sources, such as methyl bromide, which is another important ODS.

In spite of these similarities between N_2O and previously recognized ODSs and in spite of the recognition of the impact of N_2O on stratospheric ozone, N_2O has not been considered to be an ODS in the same sense as chlorine- and bromine-containing source gases. N_2O is an ODS on the basis of the extent of O_3 depletion it causes. Indeed, current anthropogenic ODP-weighted N_2O emissions are the largest of all the ODSs and are projected to remain the largest for the rest for the twenty-first century.

Some important factors that influence the ODP of N_2O have been examined. At midlatitudes, chlorine-catalyzed ozone destruction contributes most to depletion in the lowest and upper stratospheres, that is, below and above the ozone maximum. N_2O contributes most to ozone depletion just above where ozone concentrations are the largest. This leads to efficient ozone destruction from NO_x. The ODP of N_2O is lower than that of CFCs primarily because only about 10% of N_2O is converted to NO_x, whereas the CFCs potentially contribute all their chlorine.

Anthropogenic N_2O emissions are considered the single most important of the anthropogenic ODS emissions today. For example, the global anthropogenic emission of N_2O now (produced mainly as a by-product of fertilization, fossil fuel combustion and industrial processes, biomass and biofuel burning, and a few other processes) is roughly 10 million metric tons per year compared with slightly more than a million metric tons from all CFCs at the peak of their emissions. Currently, anthropogenic N_2O emissions represent the largest contribution to O_3-depleting gas emissions.

8.4 OZONE

8.4.1 Sources of Ozone

By far the most important source of O_3 contributing to atmospheric pollution is that found in photochemical smog. As discussed in the section on NO_x, disruption of the photolytic cycle of NO_2 (Equations 8.6 to 8.8) by atmospheric hydrocarbons is the principal cause of photochemical smog,

$$NO_2 \leftrightarrow NO + O \qquad (8.9)$$

$$O + O_2 \leftrightarrow O_3 \qquad (8.10)$$

$$\text{Net: } NO_2 + O_2 \leftrightarrow NO + O_3 \qquad (8.11)$$

In Equations 8.9 to 8.11, theoretically the back reaction proceeds faster than the forward reaction, so that the resulting O_3 should be removed from the atmosphere. However, free radicals formed from hydrocarbons (e.g., RO_2) and other species occurring in the urban atmosphere react with and remove NO, thus stopping the back reaction. Consequently, O_3 builds up. A large number of free radicals occur in the atmosphere, such as hydroxy radical ($OH\cdot$), hydroperoxy radical ($HO_2\cdot$), atomic oxygen [$O(^1D)$], and higher homologs $RO\cdot$ and $RO_2\cdot$, where R represents a hydrocarbon group. Free radicals participate in chain reactions, including initiation, branching, propagation, and termination reactions in the atmosphere. The $OH–HO_2$ chain is particularly effective in oxidizing hydrocarbons and NO. Some examples illustrating these reactions are the following:

$$OH\cdot + RH \rightarrow R\cdot + H_2O \qquad (8.12)$$

$$R\cdot + O_2 \rightarrow RO_2\cdot \qquad (8.13)$$

$$RO_2\cdot + NO \rightarrow RO\cdot + NO_2 \qquad (8.14)$$

$$RO\cdot + O_2 \rightarrow R'CHO + HO_2\cdot \qquad (8.15)$$

$$HO_2\cdot + NO \rightarrow NO_2 + OH\cdot \qquad (8.16)$$

As shown in these equations, the process starts with an OH· radical. After one pass through the cycle, two molecules of NO are oxidized to NO_2. The OH· radical formed in the last step (Equation 8.13) can start the cycle again. O_3 may also be formed from reactions between O_2 and hydrocarbon free radicals:

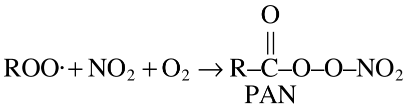
$$R\cdot \xrightarrow{O_2} RO_2\cdot \xrightarrow{O_2} O_3 + RO\cdot \tag{8.17}$$

8.4.2 Photochemical Smog

Hydrocarbon free radicals (e.g., $RO_2\cdot$) can react with different chemical species, including NO, NO_2, O_2, O_3, and a variety of hydrocarbons:

$$ROO\cdot + NO \rightarrow RO\cdot + NO_2 \tag{8.18}$$

The hydrocarbon free radicals can react with O_2 and NO_2 to produce peroxyacyl nitrate (PAN), another important air pollutant:



$$ROO\cdot + NO_2 + O_2 \rightarrow \underset{\text{PAN}}{R\text{–}\overset{\overset{\displaystyle O}{||}}{C}\text{–}O\text{–}O\text{–}NO_2} \tag{8.19}$$

or

$$RO_3 + NO_2 \rightarrow RO_3NO_2 \tag{8.20}$$

It can be seen from these equations that a large number of chemical reactions occur in the atmosphere, resulting in the formation of many secondary air pollutants. In areas such as Los Angeles, which is characterized with unique topographical conditions and abundant sunshine, these air pollutants accumulate, forming smog. Principal components of photochemical smog are O_3 (up to 90%); NO_x (mainly NO_2, about 10%); PAN (0.6%); free-radical oxygen forms; and other organic compounds, such as aldehydes, ketones, and alkyl nitrates (Table 8.2) (NAS/NRC 1977). Air pollution problems such as those found in Los Angeles and Mexico City are common among other large cities of the world.

8.4.3 Effects on Plants

Extensive studies have been conducted on the effect of O_3 on higher plants. Some examples include increase or decrease in plant growth; decrease in size, weight, and number of fruits; decrease in shoot and root growth (Henderson and Reinert 1979; Grunwald and Endress 1984); decrease in seed oil (Henderson and Reinert 1979); decrease in growth ring size (Letchworth and Blum 1977); decrease in net photosynthesis (McLaughlin et al. 1982); decrease in unsaturated fatty acids (Blum et al. 1983); increase in membrane permeability (Perchorozicz and Ting

TABLE 8.2
Compounds Observed in Photochemical Smog

Compound	Typical (or Maximal) Concentration Reported (ppm)
Ozone (O_3)	0.1
PAN ($CH_3COO_2NO_2$)	0.004
Hydrogen peroxide (H_2O_2)	(0.18)
Formaldehyde (CH_2O)	0.04
Higher aldehydes (RCHO)	0.04
Acrolein (CH_2CHCHO)	0.007
Formic acid (HCOOH)	(0.05)

Source: Adapted from NAS/NRC. *Ozone and other photochemical oxidants*. Committee on Medical and Biologic Effects of Environmental Pollutants, National Academy of Sciences, Washington, DC, 1977.

1974; Pauls and Thompson 1981); increase in respiration; and altered intermediary metabolism.

The effect of O_3 on plant metabolism is complex. However, it is well established that photochemical oxidants such as O_3 and PAN can oxidize –SH groups found in proteins, and such oxidation can inhibit enzyme activity. O_3-induced inhibition of several enzymes is involved in carbohydrate metabolism, such as phosphoglucomutase and glyceraldehyde-3-phosphate dehydrogenase. The hydrolysis of reserve starch in cucumber, bean, and monkey flower was inhibited by exposure to 0.05 ppm O_3 for 2 to 6 hours (Pauls and Thompson 1981), suggesting an inhibitory effect on amylase or phosphorylase. While decrease in glyceraldehydes-3-phosphate dehydrogenase activity suggests inhibition of glycolysis, an increase in the activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase reported by some workers implies elevated activity of the pentose phosphate pathway (Tingey et al. 1975). Studies in our laboratory indicated that mung bean seedlings exposed to 0.25 ppm of O_3 for 2 hours exhibited markedly inhibited invertase activity (M.-H. Yu, unpublished data, 2004).

O_3 exposure also interferes with lipid metabolism. For instance, lipid synthesis, requiring NADPH and ATP (adenosine triphosphate), is known to proceed at a lower rate. This may be because O_3 lowers the total energy of the cell. O_3 also causes ozonization of fatty acids. When O_3 reacts with a polyenoic fatty acid, for example, H_2O_2 and malonaldyde are produced. The structures of amino acids and proteins are also altered when these substances are exposed to O_3. Amino acids such as methionine, tyrosine, cysteine, and tryptophan are oxidized when exposed to O_3. With methionine, the oxidation leads to methionine sulfoxide formation in a concentration-dependent manner (Mudd et al. 1969; Tingey et al. 1975).

8.4.4 EFFECTS ON ANIMALS AND HUMANS

Ozone is not usually emitted directly into the air, but at ground level is created by a chemical reaction between oxides of nitrogen (NO_x) and volatile organic compounds (VOC) in the presence of sunlight. Ozone has the same chemical structure whether it occurs miles above the earth or at ground-level and can be "good" or "bad," depending on its location in the atmosphere. In the earth's lower atmosphere, ground-level ozone is considered "bad." It is the primary constituent of smog. Sunlight and hot weather cause ground-level ozone to form in harmful concentrations. "Good ozone" occurs naturally in the stratosphere approximately 10 to 30 miles (16 to 48 km) above the earth's surface and forms a layer that protects life on earth from the sun's harmful rays.

Ground-level ozone and other photochemical oxidants can cause irritation of the respiratory tract and the eye. The threshold limit value (TLV) for O_3 in industry is 0.1 ppm. Exposure to 0.6 to 0.8 ppm O_3 for 60 minutes leads to headache, nausea, anorexia, and increased airway resistance. Exposure of laboratory animals to 0.7 to 0.9 ppm may predispose or aggravate the response to bacterial infection. Coughing, chest pain, and a sensation of shortness of breath were shown in the exposed subjects who exercised (Bates and Hazucha 1973). Morphological and functional changes occur in the lung in experimental animals subjected to prolonged O_3 exposure. Such changes as chronic bronchitis, bronchiolitis, and emphysematous and septal fibrosis in lung tissues have been shown in mice, rabbits, hamsters, and guinea pigs exposed daily to O_3 at concentrations slightly above 1 ppm. Thickening of terminal and respiratory bronchioles was the most noticeable change. For example, in the small pulmonary arteries of rabbits exposed to O_3, the walls were thicker and the lumens were narrower than those of the controls. Mean ratios of wall thickness to lumen diameter were 1:4.9 for the control and 1:1.7 for the exposed animals (Bates and Hazucha 1973). This shows that the width of the lumen of exposed animals became about one-third that of the control.

As noted in Chapter 7, emphysema is a disease in which the alveoli in the lungs become damaged. The disorder causes shortness of breath and, in severe cases, can lead to respiratory or heart failure. In humans, emphysema is caused mainly by cigarette smoking, but atmospheric O_3 and some other pollutants are considered to be predisposing factors. Inhaled O_2 is passed into the bloodstream through the thin walls of alveoli and into the bloodstream, and CO_2 is removed from the capillaries to be breathed out. Tobacco smoke and other air pollutants are believed to cause emphysema by provoking the release of chemicals within the alveoli that damage the alveolar walls. As the disease progresses, the alveoli burst and form fewer, larger sacs with less surface area, so O_2 and CO_2 exchange is impaired.

Other physiological effects include dryness of upper airway passages, irritation of mucous membranes of nose and throat, bronchial irritation, headache, fatigue, and alterations of visual response.

Evidence suggests that O_3 exposure accelerates aging processes. Some investigators indicated that aging is due to irreversible cross-linking between macromolecules, principally proteins and nucleic acids. Animals exposed to 0.1 ppm O_3 may have increased susceptibility to bacterial infections. Exposed mice may have congenital abnormalities and neonatal deaths.

Development of hyperreactivity following O_3 exposure in humans and dogs has been shown. The most characteristic toxic effect of exposure to relatively high levels of O_3 is pulmonary edema (P'an et al. 1972), a leakage of fluid into the gas exchange parts of the lung. This effect was seen at concentrations only slightly above that observed in community pollution in Los Angeles, California.

Humans and animals have been shown to develop *tolerance* to O_3. Tolerance refers to increased capacity of an organism that has been preexposed to a chemical agent, such as an oxidant, to resist the effects of later exposures to ordinarily lethal, or otherwise injurious, doses of the same agent. Rodents exposed to 0.3 ppm O_3, for example, would become "tolerant" to subsequent exposures of several parts per million O_3, which would produce massive pulmonary edema in animals exposed for the first time. Some human subjects exposed to 0.3 ppm O_3 at intervals of a day or so showed diminished reactivity with later exposures. This response is designated as *adaptation* (Horvath et al. 1981).

8.4.5 BIOLOGICAL EFFECTS

A large volume of literature has been published describing the biochemical effects of O_3. Some examples include (a) reactions with proteins and amino acids; (b) reactions with lipids; (c) formation of free radicals; (d) oxidation of sulfhydryl (–SH) compounds and pyridine nucleotides; and (e) production of more or less nonspecific stress, with the release of histamine.

As mentioned in the previous section, O_3 interacts with proteins and some amino acids, altering their characteristics. In humans, the amount of lysozyme in tears of individuals exposed to smog was shown to be 60% less than normal. The concentrations of protein and nonprotein sulfhydryls in the lungs of rats exposed to 2 ppm O_3 for 4 to 8 hours were shown to be decreased. A number of investigators have shown that O_3 could cause the oxidation of the –SH group, and that addition of SH compounds was protective.

The activities of several enzymes are either enhanced or depressed in animals exposed to O_3. Reports on decreases in enzyme activities include glucose-6-phosphate dehydrogenase, glutathione reductase, and succinate-cytochrome c reductase in the lungs of rats exposed to 2 ppm O_3 for 4 to 8 hours, whereas increased activities were shown with glucose-6-phosphate dehyrogenase, 6-phosphogluconate dehydrogenase, and isocitrate dehydrogenase.

Balchum et al. (1971) have provided evidence to support the concept that the peroxidation or ozonization of unsaturated fatty acids in biological membranes is a primary mechanism of the deleterious effects of O_3. The hypothesis was based on the tendency of O_3 to react with the ethylene groups of unsaturated fatty acids, resulting in the formation of free radicals. The free radicals can, in the presence of molecular oxygen, cause peroxidation of unsaturated fatty acids. It has been observed that lipid material subjected to O_3 exposure showed a relative decrease in unsaturated fatty acids as compared to saturated fatty acids, and the more unsaturated the fatty acids were, the greater was the decrease that occurred. In addition, a deficiency of vitamin E increases the toxicity of O_3 for the rat (Goldstein et al. 1970). Possible mechanisms for O_3 toxicity involving peroxidation of membrane unsaturated fatty

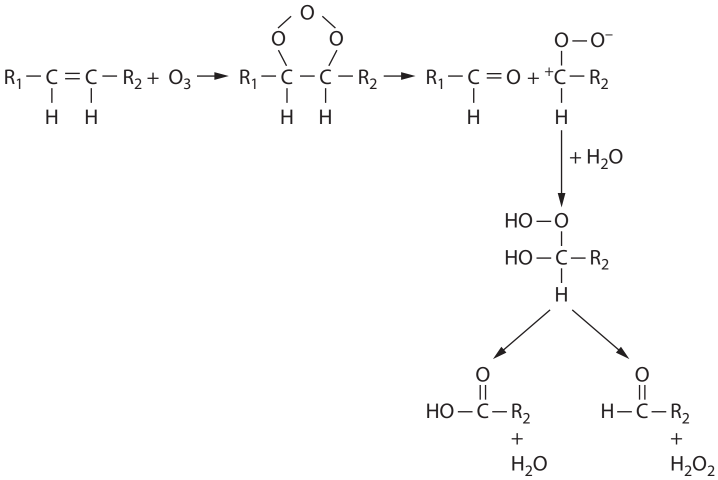


FIGURE 8.8 Ozonization of membrane lipids.

acids include (a) the ability of O_3 to react with polyunsaturated fatty acids (PUFAs), causing lipid breakdown, and some of the breakdown products can include H_2O_2, carbonyl compounds, and various free radicals that are detrimental to cells; and (b) the resultant free radicals may react with protein–SH groups, leading to inactivation of enzymes; mitochondrial PUFA, causing the swelling of mitochondria and impaired or loss of energy metabolism; lysosomal PUFA, with the release of lysosomal hydrolases; and nuclear PUFA, leading to carcinogenesis (Mueller and Hitchcock 1969).

Another chemical pathway that can induce O_3-dependent oxidation of unsaturated fatty acids is through incorporation of O_3 into the fatty acid double bond, resulting in ozonide formation (Zeevaart 1976). This process is generally known as ozonolysis (Figure 8.8). O_3 is also known to oxidize the tripeptide glutathione (GSH) and pyridine nucleotides NADH (nicotinamide adenine dinucleotide) and NADPH. The ozonization of the nicotinamide ring of NADPH may proceed in such a way as that shown in Figure 8.9.

Since the intracellular ratios of NADH/NAD$^+$, NADPH/NADP$^+$, and ATP/adenylates are carefully regulated by the cell, loss of the reduced nucleotide can be compensated by faster operation of the TCA (tricarboxylic acid) cycle. However, the cell can only make up for a net loss of all nucleotides by an increase in synthesis. The oxidation of NADH or NADPH results in elevated enzyme activity, and this permits the cell to restore the initial ratio of the nucleotides. With NADPH, oxidation increases the activity of the pentose phosphate pathway. Such increase also occurs following the oxidation of GSH (Reaction 8.21). Oxidation of either NADPH or GSH therefore may be responsible for the apparent increase in enzymes in the pentose phosphate pathway after repeated exposure to O_3.

$$2\text{GSH} + [\text{O}] \xrightarrow{\text{GSH peroxidase}} \text{GSSG} + H_2O \qquad (8.21)$$

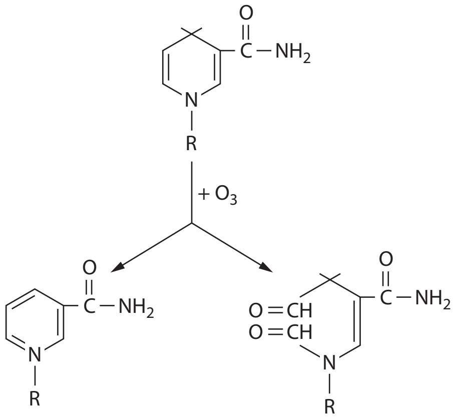


FIGURE 8.9 Ozonization of the nicotinamide ring in NADPH.

$$2\text{SSG} + 2\text{NADPH} \xrightarrow{\text{GSH reductase}} 2\text{GSH} + 2\text{NADP}^+ \tag{8.22}$$

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-P dehydrogenase}} \text{6-PG} + \text{NADPH} \tag{8.23}$$

8.5 CARBON MONOXIDE

8.5.1 Introduction

Carbon monoxide (CO) is an odorless, colorless, and tasteless gas found in high concentrations in the urban atmosphere. No other gaseous air pollutants with such a toxic potential exist at such high concentrations in urban environments. Historically, early exposures began with the use of wood fires and then coal for domestic heating. Combustion of fossil fuel associated with developing industry, explosions, fires in mines, and illumination gas prepared from coal all have been reported as sources of exposure. The migration of agricultural populations to cities increased the proportion of exposed population, as well as the number of persons generating CO. With the emergence of automobiles propelled by the internal combustion engine, the CO emitted from the exhaust pipe has become the major source for human exposure. Serious problems exist with occupational exposure to increased ambient CO for various occupations.

8.5.2 Formation

Carbon monoxide is usually formed through one of the following three processes:

1. Incomplete combustion of carbon or carbon-containing compounds. This occurs when the available oxygen is less than the amount required for complete combustion in which CO_2 is the product or when there is poor mixing of fuel and air, as shown in Equations 8.24 and 8.25:

$$2C + O_2 \rightarrow 2CO \tag{8.24}$$

$$2CO + O_2 \rightarrow 2CO_2 \tag{8.25}$$

2. Reactions between CO_2 and carbon-containing materials at high temperature. Carbon monoxide is also produced when CO_2 reacts with carbon at an elevated temperature. Such reactions are common in many industrial devices, including blast furnaces:

$$CO_2 + C \rightarrow 2CO \tag{8.23}$$

The CO produced in this way is utilized in a variety of industrial facilities, such as the blast furnace of a smelter, where CO acts as a reducing agent in the production of iron from Fe_2O_3 ores. Some CO may escape into the atmosphere, however.

$$3CO + Fe_2O_3 \rightarrow 2Fe + 3CO_2 \tag{8.26}$$

3. Dissociation of CO_2 at high temperatures. Carbon dioxide dissociates into CO and O at high temperatures, as shown in Equation 8.27:

$$CO_2 \xrightarrow{\text{high temp.}} CO + O \tag{8.27}$$

8.5.3 HUMAN EXPOSURE

CO is a poisonous and odorless gas that cannot be seen or smelled and that can kill a person in minutes if the concentration of the gas is high enough. CO is produced whenever any combustible fuel (e.g., gas, oil, kerosene, wood, or charcoal) is burned. Health effects are headache, nausea, and drowsiness that make you think you should just rest and "take it easy for a day or two." But, if the CO exposure continues, one can go into a coma and die.

A family unintentionally endangered family members and neighbors by burning charcoal briquettes indoors to keep their apartment warm (www.edmondsbeacon.com, December 17, 2009). Backdrafts, plugged flues and vents, faulty appliances, and unsafe use of outdoor grills can lead to similar poisoning accidents during cold weather and power outages.

The following are several examples showing the danger of CO poisoning: In the state of Washington in 2008, CO gas killed 64 people; 46 people were hospitalized. Nine people were taken to the hospital early Sunday, December 13, 2009, after a charcoal grill was used to heat an apartment unit in Lynnwood, Washington. Fire and aid units were called to the 59 block of 204th Street SW early that Sunday after a 3-year-old boy woke up making noises and struggling to breathe.

8.5.4 HEALTH EFFECTS

A constant supply of O_2 is needed for physiological functions to proceed normally in the body. Oxygen is carried to body tissue by hemoglobin (Hb), a complex component of the blood that consists of two pairs (α and β chains) of proteins, which themselves are bonded around an iron. Hemoglobin picks up O_2 in the lungs, forming a complex called oxyhemoglobin (HbO_2) as follows:

$$\mathrm{Hb} + \mathrm{O_2} \rightarrow \mathrm{HbO_2} \tag{8.28}$$

Once it reaches the body tissues, HbO_2 releases the bound O_2 to be used:

$$\mathrm{HbO_2} \rightarrow \mathrm{Hb} + \mathrm{O_2} \tag{8.29}$$

The Hb then returns to the lungs for a new supply of O_2.

Carbon monoxide is toxic because it enters the bloodstream and reduces the delivery of oxygen to the body's organs and tissues. The toxic action of CO involves the formation of carboxyhemoglobin (COHb or HbCO):

$$\mathrm{CO} + \mathrm{Hb} \rightarrow \mathrm{COHb} \tag{8.30}$$

The chemical affinity of CO for Hb is more than 200 times greater than that of O_2. Furthermore, in the presence of CO, HbO_2 readily releases the bound O_2 and picks up CO to form HbCO:

$$\mathrm{HbO_2} + \mathrm{CO} \rightarrow \mathrm{COHb} + \mathrm{O_2} \tag{8.31}$$

Since the hemoglobin molecule cannot be occupied by O_2 and CO at the same time, it is apparent that CO can tie up a substantial quantity of Hb in the blood when HbCO is formed. Consequently, Hb will not be able to transport O_2 to the tissues, thus severely impairing bodily functions, especially of the heart and O_2 to the central nervous system (CNS).

Although increase in the Hb oxygen concentrations can shift the equilibrium in Equation 8.29 to the left, recovery of Hb is slow, while the asphyxiating effect of putting Hb out of business is rapid. People with cardiovascular disease, particularly those with angina or peripheral vascular disease, are much more susceptible to the health effects of CO. Furthermore, research showed that the fetus is particularly susceptible to lack of O_2 supply. Thus, maternal CO poisoning during pregnancy can lead to fetal death. Animal studies showed that the offspring of pregnant female rats exposed to CO had lower birth weights and significant learning deficit (Mactutus and Flechter 1984).

The normal or background level of blood COHb is about 0.5%. Part of the CO in HbCO is derived from the ambient air, while the rest originates from the body as a result of heme catabolism. The equilibrium percentage of COHb in the bloodstream of a person continually exposed to an ambient air CO concentration of less than 100 ppm can be calculated from the following equation:

$$\text{COHb in blood (\%)} = 0.16 \times (\text{CO concentration, in the air in ppm}) + 0.5 \tag{8.32}$$

According to available data (Table 8.3), the concentration of COHb in the blood required to induce a decreased O_2 uptake capacity is approximately 5%. Impairment

TABLE 8.3
Hunan Health Effects Associated with Carboxyhemoglobin (HbCO) Levels

HbCO Levels (%)	Health Effects
<1.0	No apparent effect
2–4	Impairment of visual function; decreases in the relation between work time and exhaustion in exercising young healthy adults
2.0–4.5	Decrease in exercise capacity in patients with angina
<5	Vigilance decrement
5–5.5	Decrease in maximum oxygen consumption and exercise in young healthy men during strenuous exercise
5–17	Impairment of visual perception, of manual dexterity, of learning ability or performance of certain intellectual tasks
20–25	Nausea, weakness (particularly in the legs), occasional vomiting

Source: Pereira, L.A., D. Loomis, G.M.S. Conceijo, A.L.F. Braga, R.M. Arcas, H.S. Kishi, J.M. Singer, G.M. Bohm, and P.H.N. Saldiva. Association between air pollution and intrauterine mortality in São Paulo, Brazil. *Environ. Health Perspect.* 106, 325, 1998.

in the ability to correctly judge slight differences in successive short time intervals has been observed at lower COHb levels of 3.2% to 4.2%. The well-known symptoms of CO poisoning are headache and dizziness at COHb levels between 10 and 3,094. At levels above 30%, the symptoms are severe headache, cardiovascular symptoms, and malaise. Above COHb levels of about 40%, there is considerable risk of coma and death. In case of acute CO poisoning, 100% oxygen is commonly used to rescue the victim. The half-life of COHb is estimated to be 4 hours at rest in room air, and it is shortened to 60 to 90 minutes if 100% oxygen is given using a face mask.

In addition to binding Hb in circulating blood, CO binds other proteins in the body, including myoglobin, cytochrome c oxidase, and cytochrome P450. By binding these substances, CO impairs their action. Furthermore, CO also inhibits alveolar macrophage function, thus weakening tissue defenses against airborne bacterial infection.

Poisoning by CO is the most common form of gaseous poisoning. The poisoning occurs as a result of CO combining with Hb to form a stable COHb. This leads to reduction of the oxygen-carrying capacity of the blood, leading to tissue hypoxia. CO asphyxiation has been documented from the the time of ancient Rome. CO was discovered scientifically in 1799, and the first CO poisoning was reported in 1842. Once, accidental acute poisoning in coal mines was the main cause of illness and death among mine workers. It occurs widely among workers in a smelting furnace or boiler room and those working in a garage with its doors closed.

Despite the declines in the death rate from unintentional CO poisoning in the United States in the 1980s, CO intoxication is still common. According to the Centers for Disease Control and Prevention (CDC 2011), an estimated 10,000 persons seek medical attention or miss at least 1 day of normal activity annually because of the syndrome. In addition, 800 to 1,000 deaths occur each year, making it the most common cause of unintentional poisoning death in the United States.

Hampson et al. (1994) studied the characteristics of a series of patients poisoned with CO resulting from indoor burning of charcoal briquettes. They reported that, of 509 patients treated for acute unintentional CO poisoning, 79 cases occurred in 32 incidents as a result of indoor burning of charcoal briquettes for the purpose of either home heating or cooking. The authors suggested that all cases are avoidable, and public awareness of the risk should be enhanced.

Human exposure to CO occurs mainly from such sources as (a) CO in the surrounding ambient environment emitted from exhaust gases such as those from automobiles and industrial machinery; (b) accidental intoxication through house fires, which may contain more than 50,000 ppm CO, and environmental problems in the house, such as defective furnaces, charcoal burning in poorly vented houses, or garages connected to living quarters; (c) occupational exposure such as of firefighters (>10,000 ppm CO), traffic police, coal miners, coke oven and smelter workers, toll booth attendants, and transportation mechanics; and (d) cigarette smoking.

Precaution measures are as follows:

- Install a CO detector near all sleeping areas in your home. If the alarm sounds, leave your home immediately and call 911.
- Do not use a generator, charcoal grill, camp stove, or other gasoline- or charcoal-burning device inside your home, basement, or garage. If you are outside, do not use any of these near a home window or under a tent.
- Do not use a gas range or oven to heat a home.
- Do not run a car or truck inside a garage attached to your house, even if you leave the door open.
- Do not burn anything in a stove or fireplace that is not vented or may be clogged.
- Do not run a generator, pressure washer, or any gasoline-powered engine inside a basement, garage, or other enclosed structure, even if the doors or windows are open, unless the equipment is professionally installed and vented.
- Do call 911 if you suspect CO poisoning and are feeling dizzy, light-headed, or nauseous.
- Do have your heating system, water heater, and any other gas, oil, or coal-burning appliances checked by a qualified technician every year.
- Do keep vents and flues free of debris, especially if winds are high; flying debris can block ventilation lines.

REVIEW QUESTIONS

1. Explain the chemical changes that occur once SO_2 is absorbed into a plant leaf.
2. What could be the basis for different plant species to exhibit different sensitivity to SO_2?
3. How is SO_2 exposure related to the respiratory system in animals and humans?
4. Briefly explain the role that the free hydroxyl radical (OH) plays in the atmosphere.

5. Describe the photolytic cycle of NO_2.
6. Explain the way in which plants may make use of dissolved NO_x.
7. Describe how N_2O may be formed.
8. Explain the importance of N_2O in relation to stratospheric O_3 layer depletion.
9. Explain how O_3 may accumulate in the troposphere.
10. What is photochemical smog? What are the main components?
11. Explain how O_3 may injure membrane structure.
12. What is PAN? Explain how it may be formed.
13. What is the most noticeable change in the bronchioles of animals exposed to O_3?
14. What is pulmonary edema? Which air pollutant(s) can cause it? SO_2; NO_2; F; O_3.
15. Complete the following reaction: $2GSH + [O]$ (from O_3) $\rightarrow$.
16. What is ozonolysis?
17. Explain how CO may be formed from CO_2.
18. What is carboxyhemoglobin? Write a chemical equation to show its formation.
19. What is the physiological basis for the toxicity of CO?
20. Explain how CO may be related to macrophage function.

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9 Air Pollution

Particulate Matter

9.1 INTRODUCTION

This chapter discusses two of the six common air pollutants—particulate matter and lead. Particulate matter is examined in Section 9.1 through Section 9.7, whereas lead is presented in Section 9.8. Particle pollution, also called particulate matter or PM, refers to a mixture of solid particles and liquid droplets found in the air. The physical dimensions and chemical properties of these aerosols vary greatly. Their size may vary from 0.5 to 10^{-7} mm, and they are composed of a large number of inorganic and organic materials, including metals and nonmetal elements (and their oxides, nitrates, and sulfates). Although it is often convenient to group them as *particulates*, their sources, distribution, and effects can be highly variable. Because of the large quantities of particulates emitted into the atmosphere from different sources, and the potential adverse effects they can cause, the U.S. Environmental Protection Agency (EPA) has designated particulate matter (PM) as one of the six criteria air pollutants to be regulated. In 1987, the agency added a new standard for particulates called PM_{10} (referring to PM with diameter less than 10 μm), based on the evidence that the smaller PM has the greatest impact on health because of its capacity to be inhaled. This chapter presents an overview of this class of air pollutants, followed by discussion of three specific examples of PM: silica, beryllium, and asbestos.

9.2 CHARACTERISTICS OF PARTICULATE MATTER

Particulates are usually classified into primary or secondary. Primary particulates are larger (usually 1 to 20 μm in diameter) and are emitted directly into the atmosphere by a variety of chemical and physical processes. Secondary particulates are relatively smaller and are formed through chemical reactions that occur in the atmosphere (Fennelly 1976). The composition of particulates varies from place to place and includes thousands of entities that differ in size, surfaces, and toxicity (Abelson 1998). Particles in most urban aerosols have been shown to contain a number of potentially toxic trace chemical species, such as lead (Pb), cadmium (Cd), nickel (Ni), selenium (Se), vanadium (V), zinc (Zn), cobalt (Co), manganese (Mn), bromine (Br), sulfate, and benzo[a]pyrene (Natusch and Wallace 1974).

Evidence from recent studies strongly suggests the importance of the primary urban aerosols. It is considered that, although these primary aerosols contribute minor amounts of the whole aerosol mass, they serve as condensation nuclei on which the secondary aerosol mass resides, and they carry the bulk of the particulate toxin and aerosol particles (Ondov and Wexer 1998).

9.3 FORMATION OF PARTICULATES

Particulates (PM) are formed from both natural and anthropogenic sources. Natural sources include volcanic ash, wildfire particles, fine soil particles, fine marine salts from ocean sprays, and biological particles such as pollen, fungal spores, and others. Fine particulates are also produced in air as a result of atmospheric reactions, such as through photochemical reactions. Anthropogenic sources include a variety of industrial combustion processes, mining, vehicle emissions, domestic heating and cooking, pottery making, metalworking, and many other manufacturing processes. In addition, cultivation of agricultural lands also contributes significant amounts of PM through land clearance and fire control activities.

9.3.1 Physical Processes

Participate matter can be formed through both physical and chemical processes. Particles above approximately 1 mm in size are generally formed by the disintegration of larger particles. This is called a *dispersion process*, and the product is known as a *dispersion aerosol*. Dusts are solid dispersion aerosols, which may be formed through a variety of natural and human activities. Some examples include volcanic eruption, windblown dust from dry soil, ocean spray, coal grinding, rock crushing, stonecutting and polishing, high-power drilling of tunnel rocks, and manufacture of pottery.

9.3.2 Chemical Processes

Both inorganic and organic particles are produced through various chemical processes. Metal oxides make up a major class of inorganic particles in the atmosphere. They are produced whenever fuels containing metals are burned. For instance, particulate iron oxide is produced in the combustion of coal, which contains iron sulfide (FeS_2) as a contaminant:

$$3FeS_2 + 8O_2 \rightarrow Fe_3O_4 + 6SO_2 \quad (9.1)$$

As noted in Section 8.2, sulfuric acid mists are formed from the oxidation of atmospheric SO_2:

$$2SO_2 + O_2 + 2H_2O \rightarrow 2H_2SO_4 \text{ (liquid droplets)} \quad (9.2)$$

The sulfuric acid thus formed can react with basic air pollutants such as ammonia (NH_3), CaO, PbO, or $A1_2O_3$, forming various sulfates:

$$H_2SO_4 \text{ (droplet)} + 2\,NH_3 \text{ (gas)} \rightarrow (NH_4)_2SO_4 \text{ (droplet)} \quad (9.3)$$

$$H_2SO_4\text{(droplet)} + PbO \text{ (particle)} \rightarrow PbSO_4 \text{ (droplet)} + H_2O \quad (9.4)$$

Combustion of leaded gasoline results in the formation of several kinds of lead halides, among other substances. Tetraethyl lead [$Pb(C_2H_5)_4$] in leaded gasoline reacts with molecular oxygen (O_2) and halogenated scavengers, such as dichloroethane and dibromoethane, producing various forms of lead halide particles that are emitted into the atmosphere:

$$\begin{gathered}\mathrm{Pb(C_2H_5)_4} + \mathrm{O_2} + (\text{Halogenated scavengers}) \rightarrow \\ \mathrm{CO_2} + \mathrm{H_2O} + \mathrm{PbCl_2} + \mathrm{PbBrCl} + \mathrm{PbBr_2}\end{gathered} \tag{9.5}$$

In addition, whole gasoline vapor alone has been shown to contribute significantly to atmospheric aerosol formation (Odum et al. 1997).

9.4 HEALTH EFFECTS

Fly ash particles are generally composed of stable elements or compounds that are usually not considered directly toxic in concentrations found in ambient air. However, subtle toxicity has been recognized under different conditions. In particular, many trace elements have important biological activity and are therefore potential health hazards.

The toxicity of PM generally arises from any of the following factors: The particles may themselves be toxic, such as particles containing toxic metals such as lead, cadmium, nickel, and mercury (Hg) and nonmetals such as arsenic (As) and radionuclides (Costa and Mollenhauer 1980; Hauser et al. 1995). Alternatively, the particles may adsorb toxic chemicals such as carcinogens (Schwartz 1994) and enhance their effect by increasing their penetration into the lungs or by prolonging their residence time in the respiratory tract. Particles may also serve as condensation nuclei for water and other vapors, producing droplets and enhancing biological effects. Finally, if there are large quantities present in the respired air, particles may overtax the mucociliary apparatus, thus decreasing the rate of removal of toxic chemicals from the lung.

Many occupational activities cause the formation of dust. Mining, metal grinding, and sandblasting activities for varying periods of time have been shown to cause *pneumoconiosis,* a disease of the lung caused by habitual inhalation of irritant mineral or metallic particles. The disease is characterized by fibrous degeneration known as *fibrosis.* Among the several factors that contribute to the development of pneumoconiosis, those related to workers and dust are particularly important. The factors pertaining to workers include duration of exposure to dust and the workers' susceptibility.

Concerning dust, the size of particles, their chemical composition, and their concentrations are important. Researchers have identified various diseases based on the chemical elements involved in fibrosis formation. For example, *silicosis* results from inhalation of silicon oxide (SiO_2); *silicatosis,* from silicate; *siderosis,* from hematite, F_2O_3; *talcosis,* from talc; and *bariosis,* from barium (Ba).

The size of particles is important in the pathogenesis of pneumoconiosis. This is because the size affects the concentration of particles that may be suspended in the air, or it may determine the depth to which these particles penetrate into the lung and the amounts in which they may be deposited and retained.

In addition to widely recognized occupational health effects of PM, numerous epidemiological studies have confirmed that total suspended particles (TSP) present in urban areas, especially those less than 2.5 μm in diameter, were associated with increased risk of mortality in pneumonia and cardiovascular disease. The risk is particularly elevated in the elderly (Costa and Mollenhauer 1980). Available information indicated widespread acute impacts on the health of a large number of populations in Indonesia during the rain forest fire episode in the summer of 1997. According to the Singapore Ministry of Health, there was a 13% increase in visits to government clinics for acute respiratory infections and a 19% increase in asthma visits during the last week of September when PM levels peaked (Brauer and Hishum-Hashim 1998).

China has achieved marked economic growth since 1980. The growth has been followed by rapid industrialization, accelerated urbanization, and greatly increased energy consumption (He et al., 2002). The accelerated urbanization is evidenced by steady increases in the proportion of urban population to the total population, from 18% in 1978 to 31% in 1999, a growth rate three times the world average during this period. The explosive economic growth also made China the world's second-largest energy consumer after the United States. Energy consumption, especially coal consumption, is the main source of anthropogenic air pollution emissions in Chinese cities. Between 1978 and 1999, China's energy consumption more than doubled. Coal, the primary energy source in China, accounted for about 74% of the total energy consumption during this period. The increased use of coal is considered the origin of many air pollution problems, including SO_2 pollution, participate matter, and acid rain (He et al. 2002).

Xu et al. (1995) reported that coherent evidence was present that the existing air pollution levels in Beijing were associated with adverse health outcomes. The scientists studied the data on the average number of daily hospital outpatient visits at a community-based hospital in Beijing and compared the data with the levels of SO_2 and TSP in the atmosphere. They found that increases in the levels of the two types of pollutants were significantly correlated with the increases in internal medicine visits.

A similar observation was made in Seoul, South Korea, where several scientists investigated the impact of air pollution on human health. For example, Ha et al. (2003) studied the effect of air pollution on mortality among postneonates, those aged 2 to 64 years, and those over 65 years of age. The study included daily counts of total and respiratory death along with analyses of daily levels of PM_{10}. The results showed that, in terms of mortality, infants were most susceptible to PM_{10}, especially if deaths were related to respiratory system.

On August 10, 2010, the U.S. EPA finalized a regulation limiting the release of mercury and other toxic air pollutants from cement plants to save lives. It was the first time the federal government had restricted emissions from existing cement kilns. The regulations aim to reduce annual emissions of mercury and PM by 92% by 2013. EPA officials indicated that the limits would benefit children, whose brains can be damaged by mercury that makes its way through the air to water to fish that children eat. They also predicted that the rules would stave off thousands of premature heart and lung deaths each year attributed to particulate pollution (*Bellingham Herald* August 10, 2010).

9.5 SILICA

Silica (silicon dioxide, SiO_2) and silicates constitute the major portion of all rocks and their products such as soils, sands, and clays. Silicon (Si) itself is the second most abundant element (after oxygen) in Earth's crust. Silica occurs in either its free form or a combined state called *silicate*. Free silica may be in crystalline form, such as quartz, granite, flint, and diatomite or in noncrystalline form.

9.5.1 SILICOSIS

Silicosis is a disease caused by breathing tiny particles of free SiO_2. It is considered the most important of the pneumoconioses, or dust diseases of the lung, not only because of its highly damaging effect on the respiratory system but also because of the large numbers of workers throughout the world who are at risk of contracting it. Silicosis may be acute because the disease is manifested within 8 to 18 months following the first exposure. Chronic silicosis may develop with a latency period up to 20 years and is found among people engaged in mining industries, pottery manufacture, stonecutting and polishing, tile and clay production, and glass manufacture. Silicosis increases susceptibility to various respiratory infections, notably tuberculosis.

The size of offending silica particles is extremely important in determining the degree of tissue reaction that will occur following the inhalation of the siliceous dust. Particles of silica or silicate from 0.5 to 10 μm are responsible for the disease because they lead to fibrogenic reaction in alveolar tissue. Fibrous, or scar, tissue is formed to replace the normal lung tissue. But, the fibrous tissue does not have the elasticity of normal tissue and handicaps the lung in performing its ventilatory function and the exchange of gases between the air and blood. As a result, the victim becomes short of breath, a principal clinical characteristic of silicosis.

9.5.2 PATHOGENESIS

Many hypotheses have been advanced to explain the mode of action of silica pertaining to its fibrogenic properties. For many years, researchers thought the fibrogenic properties were due to the action of silicic acid (H_4SiO_4). However, Allison et al. (1966) suggested that the intracellular reaction to silicic acid is the first of a two-stage process in which the major fibrogenic stimulus comes from the action of cellular enzymes rather than directly from silicic acid itself.

According to their hypothesis, once a silica particle is arrested in the lungs, it is invaginated, initiating phagocytosis. The particle is encapsulated within the cell in a phagosome that soon becomes converted into a second type of lysosome (digestive vacuole) through merging with primary lysosome presumably secreted by the Golgi body. Protective substances adsorbed onto the silica particles (e.g., plasma proteins) are stripped off by the enzymes released from the lysosome, exposing silicic acid. The highly reactive silicic acid acts as a hydrogen donor to form hydrogen-bonded complexes with active groups of the lipid membrane, such as phosphate ester groups, and with secondary amide (peptide) groups of proteins.

This reaction causes the lysosomal membrane to become permeable, allowing its enzymes to leak into the cytoplasm and destroy the cell. With cellular dissolution, the cell contents, including active lysosomal enzymes, along with the ingested silica, are released into the tissue interstices, as illustrated in Figure 9.1. Moreover, the freed particles of silica are again phagocytosed by other macrophages, leading to the chain of events.

9.6 BERYLLIUM

Beryllium (Be) is one of the least-known environmental pollutants, yet it is one of the most toxic nonradioactive elements known. Its industrial *threshold limit value* (TLV) is 2 mg/m^3, the lowest of all particulates. (The TLV of a toxicant is defined as the maximum concentration to which it is believed healthy workers may be repeatedly exposed without ill effect based on an 8-hour working day.) The toxicity of beryllium disease in humans was described in the United States in the 1940s, when more than 500 cases were reported. Acute disease occurred in Ohio among beryllium extraction and production workers, while chronic disease was found in Massachusetts among workers manufacturing fluorescent lamps containing beryllium phosphor. Because of the extreme toxicity of beryllium, its use in fluorescent lamps was discontinued in 1950. As a result, beryllium disease incidence has decreased dramatically.

Beryllium is estimated to comprise about 0.0006% of Earth's igneous rocks. Of 28 minerals in which beryllium is a minor accessory constituent, only *beryl* or beryllium aluminum silicate ($Be_3Al_2Si_6O_{16}$), with 14% BeO, is the chief source of beryllium and as such is the most important commercially. Beryllium is also found in coal in amounts ranging from 0.1 to 1.000 ppm.

9.6.1 SOURCES OF EXPOSURE TO BERYLLIUM

Beryllium compounds can occur within the production and manufacturing industries as well as in housekeeping, maintenance, salvage, and solid waste areas. Individuals working in all operations involving the production of airborne beryllium are at risk for developing beryllium disease. Major occupations at risk for developing beryllium disease have changed since the first reports of cases in the United States in the 1940s. Before 1950, common exposure occurred in fluorescent lamp manufacturing, atomic bomb research, and beryllium extraction operations. After 1950, beryllium was replaced by a calcium phosphor in fluorescent lamp production. However, beryllium has since been used in modern technology, including nuclear reactors and electronics equipment, guidance and navigation systems, rocket parts, and heat shields. It is employed extensively as an alloying agent for copper since it adds tensile strength, conductivity, and corrosion resistance.

Chronic beryllium disease has been reported in people living in areas adjacent to a plant or industry using the metal, suggesting neighborhood exposure from plant discharges into the air. It has also been shown that families of beryllium workers may also be exposed to the metal as a result of dust carried home on the workers' clothes. Between 1973 and 1980, there were 66 cases reported in the United States. Nearly

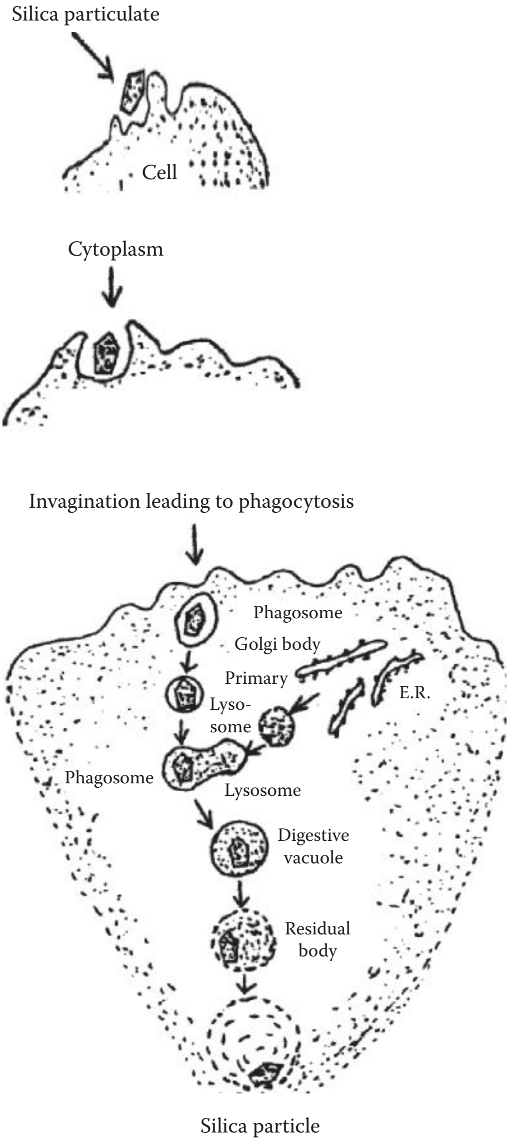
Silica particulate
Cell
Cytoplasm
Invagination leading to phagocytosis
Phagosome
Golgi body
Primary
Lyso-
some
E.R.
Phagosome
Lysosome
Digestive
vacuole
Residual
body
Silica particle

FIGURE 9.1 Disruption of cell with release of digestive enzymes and silica. E.R., endoplasmic reticulum.

half of these were invoked in beryllium metal production. The combustion of coal is considered the largest source of environmental beryllium contamination. Some coal contains about 2.5 ppm, and oil contains about 0.8 ppm beryllium. Atmospheric emission of beryllium from these sources was estimated to be above 1,000 metric tons annually (Goyer 1986).

9.6.2 HEALTH EFFECTS

Chronic beryllium disease, commonly known as berylliosis, is manifested by pulmonary and systemic granulomatous disease caused by exposure to beryllium by inhalation. The duration of exposure may be from several months to years. The interval between initial exposure and clinical manifestations of disease varies with individuals. Some patients may not become symptomatic until up to 25 years after their last exposure. The average latency is 10 to 15 years. The most common symptom of chronic beryllium disease is dyspnea (shortness of breath). Other symptoms include cough, fatigue, weight loss, chest pain, signs of pulmonary hypertension, nodular skin lesions, and conjunctivitis.

In acute disease, nasopharyngitis, tracheobronchitis, or chemical pneumonitis may occur, resulting in edema, inflammation, and necrosis. The severity of clinical disease depends largely on the dose of beryllium exposure. Symptoms and signs are nonspecific, identical to those found in any case of chemical pneumonitis secondary to a lung irritant and include dyspnea, cough, chest pain, blood-tinged sputum, and cyanosis. Acute beryllium disease is currently uncommon. Evidence from both animal experiments and human epidemiologic findings suggests a link between beryllium and lung cancer in humans.

9.6.3 BIOLOGICAL EFFECTS

In animal studies, beryllium has been shown to cause ultrastructural changes in liver. Alterations included vacuolization and dense deposits in lysosomes, loss of fibrils and appearance of dense plaques in some nucleoli, and distortion of bile canaliculi (Goldblatt 1973). Changes in lysosomal morphology were found to correlate with the biochemical evidence of localization of beryllium within lysosomes. Increases in serum λ-globulins, elevated erythrocyte sedimentation rate, and erythrocytosis, hyperuricemia, and transient hypercalcemia and hypercalciuria have also been noted.

Beryllium affects the enzyme that leads to DNA synthesis and can act as a competitive inhibitor of magnesium (Mg^{2+}), a cofactor for DNA polymerase. DNA polymerase catalyzes the formation of a polynucleotide from a single DNA template strand and a short complementary DNA or RNA primer. It also functions to “proofread” the base pairing as a new strand of DNA is formed. In this way, it can remove an incorrectly base-paired nucleotide before the next nucleotide is added to the DNA strand. Therefore, when beryllium competitively inhibits Mg^{2+}, a base substitution mutation may occur. It has been reported that the physical properties of DNA are affected by 0.1 to 1 mM beryllium sulfate ($BeSO_4$).

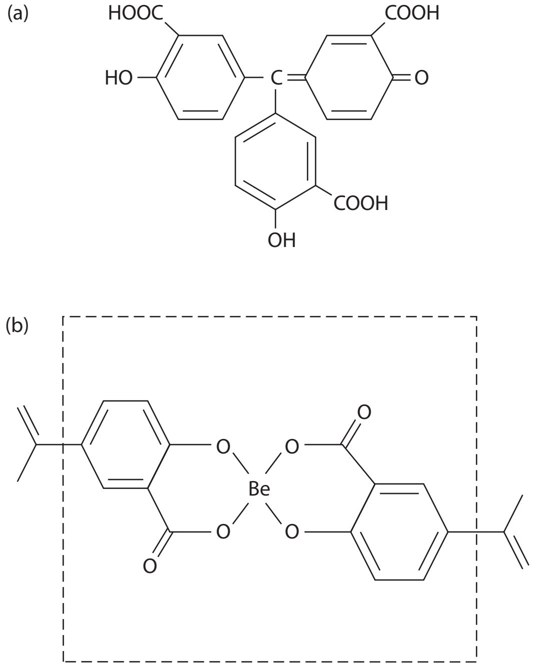

FIGURE 9.2 (a) Chemical structure of aurintricarboxylic acid (ATA). (b) Suggested mechanism by which beryllium is chelated by ATA.

9.6.4 THERAPY

One of the remedies for berylliosis is the use of chelating agents, such as antitricarboxylic acid (ATA) (Figure 9.2a). In an animal experiment, researchers injected mice with enough beryllium salt to kill them within a few days. Half of the animals were injected with a small quantity of ATA, and the other half were left untreated. The results showed that virtually every animal treated with ATA survived and continued to live normally, whereas all the untreated animals died. The experiments were repeated with hundreds of animals of different species, with the same high degree of protection. Subsequent studies using radioactive beryllium and ATA showed that the chelating agent was found in practically every cell where beryllium was present. Previously damaged cells recovered, and within a few days, they could not be distinguished from the normal tissue cells. How ATA functions chemically to antagonize beryllium is incompletely understood. A suggested mechanism involved in the chelate is shown in Figure 9.2b.

9.7 ASBESTOS

Asbestos is the generic name of a class of natural fibrous silicates. There are four commercially important forms: chrysotile, amosite, anthophyllite, and crocidolite (Public Health Service 2002). Asbestos is mined primarily from open pits. The annual consumption of asbestos in the United States peaked at about 800,000 tons in 1973, but by 1984 it declined to slightly above 200,000 tons, mainly because of concerns about its toxicity (Zurer 1985).

9.7.1 CHEMICAL AND PHYSICAL PROPERTIES

Asbestos minerals have a number of desirable properties that were useful in commercial applications. These include tensile strength, heat stability, thermal and electrical insulation, wear and friction characteristics, the ability to be woven, and resistance to chemical and biological degradation (Public Health Service 2002).

Chrysotile, the most abundant form of asbestos, occurs naturally in lengths from 1 to 20 mm. Its chemical composition is $Mg_3(Si_2O_5)(OH)_4$, and it exists as a curled sheet that forms a spiral around a central hollow tube silicate. Small amounts of iron, aluminum, nickel, calcium, chromium (Cr), manganese, sodium, and potassium may be present as impurities. Chrysotile may be white, gray, green, or yellowish, with a silky luster (Public Health Service 2002).

9.7.2 USE

Although asbestos use dates back at least 2,000 years, modern industrial use began around 1880. Asbestos demand peaked in the late 1960s and early 1970s when more than 3,000 industrial applications or products were listed. It has been estimated that in the United States more than 320,000 km of asbestos-cement pipes, carrying drinking water and other materials, had been laid. Asbestos has been used in brake linings, roofing, clutch facings, thermal and electrical insulation, cement pipes and sheets, filters, gaskets, and friction materials, textiles, steam and fire hoses, plastics, gas mask filters, paper, and other products (Public Health Service 2002).

Consumption of asbestos in the United States has been declining for three decades. Reported consumption in 1980 was 360 million kg. By 1998 and 1999, U.S. consumption had declined to about 16 million kg/year. Only chrysotile is presently used for manufacturing in the United States, most of it is used in plastics (Public Health Service 2002).

9.7.3 EXPOSURE

Asbestos is released to the environment from both natural and anthropogenic sources and has been detected in indoor and outdoor air, soil, drinking water, food, and medicines. Asbestos has been detected within the Greenland ice sheet. The primary routes of potential human exposure to asbestos are inhalation and ingestion. Worker exposure is a concern in the mining and milling of asbestos, during manufacture of all asbestos products, and in the construction and shipbuilding industries. It has been reported that in the United States an estimated 37,000 persons were employed in manufacture of primary asbestos products, while 300,000 persons were in secondary asbestos industries. In addition, workers may be exposed to asbestos in consumer industries such as brake repair, asbestos insulation, and asbestos abatement. According to a 1990 Occupational Safety and Health Administration (OSHA) report, approximately 568,000 production and service industry workers and 114,000 construction industry workers were potentially exposed to asbestos (Public Health Service 2002).

Asbestos bodies were found in 48.3% of lungs of 3,000 consecutive autopsies from three hospitals in New York. Similar observations were made in other big cities. In addition to the lungs, other organs, such as the thyroid, spleen, pancreas, heart, adrenals, kidney, prostate, brain, and liver, have also been shown to contain some asbestos.

9.7.4 HEALTH EFFECTS

Health problems related to exposure to asbestos were first observed by the early 1900s. It was recognized in the 1950s and 1960s that asbestos was responsible for lung and pleural tumors in asbestos miners (Mosman et al. 1989). The effects of asbestos fibers in biological systems may result not only from the properties of the fibers themselves but also from contamination with inorganic or organic substances that occur naturally or are added during mining, milling, processing, or shipping.

The proven or suspected effects of asbestos minerals on human health include nonmalignant changes, such as pulmonary and pleural fibrosis, and several types of malignancy, especially of the lung, pleura, and peritoneum. Association between asbestos and human disease was revealed from studies of certain occupational groups, notably workers engaged in the mining and milling of asbestos, the manufacture of asbestos-containing products, and the application and removal of asbestos-containing insulating materials.

Asbestosis (or asbestotic pneumoconiosis) may develop after years of intense exposure and was the first clearly demonstrated adverse effect of asbestos in humans. The condition is characterized by pulmonary fibrosis (scarring of the lungs). Victims become increasingly short of breath and in extreme cases may die of heart failure. Some researchers suggested that between 1950 and1975, approximately 10% of the deaths among New York City insulation workers were caused by asbestosis. Pulmonary fibrosis sufficient to interfere with respiratory or cardiovascular function can be prevented by reducing the asbestos dust concentration to levels that are still far above any likely to be encountered in community air. Calcified pleural plaques occur frequently in workers exposed to asbestos.

On the basis of sufficient evidence of carcinogenicity in humans, asbestos and all commercial forms of asbestos are now recognized as “known to be human carcinogens” (Goldblatt 1973). Occupational exposure to chrysotile, arnosite, anthophyllite, and mixtures containing crocidolite has resulted in a high incidence of lung cancer. Mesotheliomas (a neoplasm derived from cells lining the chest or abdominal cavities) have been observed after occupational exposure to crocidolite, amosite, and chrysotile asbestos. Gastrointestinal cancers occurred at an increased incidence in groups occupationally exposed to several types of asbestos. An excess of laryngeal cancer has also been observed in some groups of exposed workers. No clear excess of cancer has been associated with the presence of asbestos fibers in drinking water. Mesotheliomas have occurred in individuals living in the neighborhood of asbestos factories and mines and in people living with asbestos workers (Goldblatt 1973). As mentioned in Chapter 5, there is synergism between cigarette smoking and asbestos exposure in relation to lung cancer development.

In March 2008, the Justice Department and EPA announced that chemical maker W.R. Grace had agreed to pay $250 million to reimburse the federal government for investigation and cleanup costs of asbestos contamination in Libby, Montana. The settlement is the largest in the history of the Superfund program. W.R. Grace owned and operated a vermiculite mine in Libby from 1963 to 1990, and the vermiculite ore was contaminated with asbestos. The contamination resulted in many cases of lung disease in and around the Libby area. In addition to the cleanup lawsuit, officials of the company also face a multiple-charge federal criminal indictment filed in 2006 (www.cen.online.org 2008).

The health problems associated with asbestos exposure have been widely recognized. In the United States, occupational standards have become more stringent and testing methods more sophisticated. With improvement in remediation methods and the availability of more information about how asbestos fibers cause health problems in humans, it is hoped the threat of asbestos to humans will continue to diminish.

As is widely known, tens of thousands of firefighters and medics rushed to New York City for rescue work following the disastrous attacks on the World Trade Center on September 11, 2001. Recent studies showed that most of the rescuers whose lungs were damaged by breathing pulverized masonry and glass from the terrifying attacks are not improving. The results are based on breathing tests from nearly 13,000 rescue workers who were at Ground Zero in the first 2 weeks after the attacks, when the dust cloud was thickest. It was found that of the firefighters who did not smoke, 13% were still scoring below normal up to 7 years later. According to the report from the fire department and the Albert Einstein College of Medicine, that was down from 18% who initially tested below normal after the attacks. The firefighters were exposed to "unprecedented density of dust, smoke, all kinds of materials," according to Dr. Thomas Aldrich, professor of medicine at Albert Einstein College (*USA Today* 2010).

9.8 LEAD

9.8.1 Sources of Lead

Lead (Pb) is a metal found naturally in the environment as well as in manufactured products. The major sources of lead emissions have historically been motor vehicles and industrial sources. As a result of EPA's regulatory efforts to remove lead from motor vehicle gasoline, emissions of lead from the transportation sector have greatly declined over the last three decades. Major sources of Pb emissions to the air today are ore and metals processing and leaded aviation gasoline. The highest air concentrations of Pb are usually found near lead smelters. Other stationary sources are waste incinerators, utilities, and lead-acid battery manufacturers.

9.8.2 National Lead Emissions

According to EPA's 2005 report on national lead emissions, the two largest lead emission sources are non-road equipment (495 tons/year), and industrial processes (486 tons/year). These are followed by fossil fuel combustion, waste disposal, electricity generation, and solvent use. A later report showed great improvement observed in lead air quality between 1980 and 2009, accounting for a 93% decrease in the national average.

9.8.3 LEAD AIR QUALITY STANDARDS

The Clean Air Act established two types of national air quality standards for Pb. They are Primary standards and Secondary standards. Primary standards set limits to protect public health, including the health of "sensitive" populations such as asthmatics, children, and the elderly. The Primary standard is 0.15 μg/m³, on a rolling 3-month average. Secondary standards set limits to protect public welfare, including protection against visibility impairment, and damage to animals, crops, vegetation, and buildings. It is the same as with the Primary standard.

The Clean Air Act requires the EPA to review the latest scientific information and standards every 5 years. Before new standards are established, policy decisions undergo rigorous review by the scientific community, industry, public interest groups, the general public, and the Clean Air Scientific Advisory Committee (CASAC).

9.8.4 EFFECT OF LEAD ON HEALTH

In addition to exposure to Pb in air, other major exposure pathways include ingestion of Pb in drinking water and Pb-contaminated food as well as incidental ingestion of Pb-contaminated soil and dust. Lead-based paint remains a major exposure pathway in older homes.

Once taken into the body, Pb distributes throughout the body in the blood and is accumulated in the bones. Depending on the level of exposure, Pb can adversely affect the nervous system, kidney function, immune system, the cardiovascular system, and reproductive and developmental systems. Pb exposure also affects the oxygen carrying capacity of the blood. The Pb effects most commonly encountered in the general populations are neurological effects in children and cardiovascular effects (e.g., high blood pressure and heart disease) in adults. Infants and young children are especially sensitive to even low levels of Pb, which may contribute to learning deficits, lowered IQ, and behavioral problems.

Lead is persistent in the environment and accumulates in soils and sediments through deposition from air sources, direct discharge of waste streams to water bodies, mining, and erosion. Ecosystems near point sources of Pb demonstrate a wide range of adverse effects including losses in biodiversity, changes in community composition, decreased growth and reproductive rates in plants and animals, and neurological effects in vertebrates.

REVIEW QUESTIONS

1. Define (a) primary and (b) secondary particulates.
2. Explain the characteristics of particulate matter.

3. Why is "size" particularly important in determining the toxicity of particulate matter?
4. What are some of the natural sources of particulate matter? Anthropogenic sources?
5. Explain the importance of H_2SO_4 as particulate matter.
6. Explain the reasons for the toxicity of particulate matter.
7. What is pneumoconiosis?
8. What is silicosis? Why is silicosis important in public health/toxicology?
9. Explain the current understanding of the mode of action for silicosis.
10. Explain why combustion of coal is considered the largest source of environmental beryllium contamination.
11. How is beryllium related to DNA?
12. What is ATA? Explain how it may help alleviate beryllium toxicity.
13. List the health effects of asbestos.
14. What types of cancers are associated with asbestos exposure?
15. Explain how smoking and asbestos exposure may be related.
16. Explain the health effect of lead in general population.
17. What is the health effect of Pb in infants and young children?

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10 Environmental Fluoride

10.1 INTRODUCTION

Although fluoride is not listed as one of the criteria air pollutants regulated by the Environmental Protection Agency (EPA), it is nevertheless an important gaseous air pollutant. Fluoride (F) is the most phytotoxic air pollutant because it can damage plants at extremely low concentrations. In addition, adverse effects of F are not limited to airborne F: High levels of waterborne F are also hazardous to both humans and animals. In China and India, for example, millions of people are suffering from dental and skeletal fluorosis (abnormal or poisoned tooth and bone conditions induced by F), mainly due to consumption of high levels of F in drinking water.

In this chapter, the sources and forms of F found in our environment are presented, together with the way in which F has an impact on the health of living organisms. Reference also is provided to describe the concerns shared by several less-developed countries that have attained marked economic growth but are experiencing increasing problems with F.

10.2 OCCURRENCE AND FORMS OF FLUORIDE

10.2.1 INTRODUCTION

Fluorine (F) is the lightest element in group VII of the periodic table, with atomic number 9 and an atomic weight of 18.998. It has a single isotope, and its valence in all naturally occurring compounds is 1. F is ubiquitous: It rarely occurs free in nature, but combines with a variety of elements to form fluorides that exist in minute amounts in air, water, minerals and soils, foods, and body tissues. F found in our environment is derived from both natural and anthropogenic sources. Natural sources of F include volcanism, aerosols from ocean spray, soil dust blown into the atmosphere, and so on, while anthropogenic sources occur mostly in industrial facilities. F emitted into the atmosphere from these sources consists of both gaseous and particulate forms and as such can also contribute F to surface waters.

10.2.2 AIRBORNE FLUORIDE

Fluoride concentrations in air in U.S. residential or rural communities vary markedly, ranging from less than 0.04 to 1.2 ppb (0.03-0.90 µg F/m^3) (NAS/NRC 1971). In many cities in less-developed countries, the level is much higher. For example, in Beijing, China, the level is reported to be 0.11–2.14 ppb (0.08–1.61 µg F/m^3, average 0.61 µg/m^3) (Feng et al. 2003).

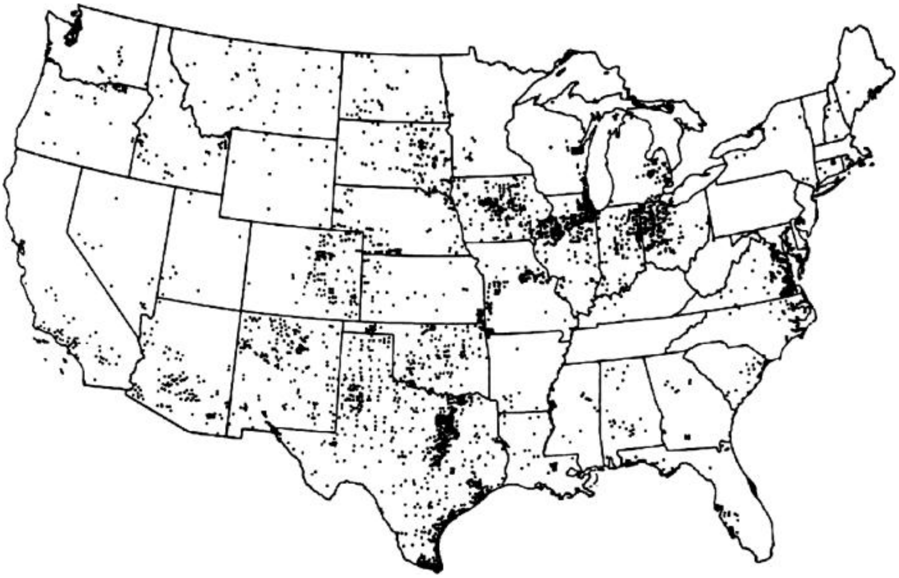


FIGURE 10.1 Distribution of communities in the United States with 0.7 ppm F or more natural fluoride in community water supply. (Adapted from NAS/NRC, NAS Subcommittee on Fluorosis. 1974. *Effects of fluorides in animals.* National Academy of Sciences, Washington, DC, 4.)

10.2.3 NATURAL WATERS

F content in natural waters in the United States ranges from 0.02 to 0.2 ppm. In 1969, there were 2,630 communities in the United States with a drinking water supply with a natural F concentration of 0.7 ppm or more (Figure 10.1) (NAS/NRC 1974). River waters contain 0.0-6.5 ppm, with an average of 0.2 ppm. Groundwaters contain from 0.1 to 8.7 ppm F, depending on the rocks from which the waters flow. The level of F in seawater is about 1.4 ppm (NAS/NRC 1971).

10.2.4 MINERALS AND SOILS

The F content in rocks is about 0.06-0.09% (by weight). The most important F-containing minerals and soils are fluorspar (CaF_2) or fluorite, cryolite (Na_3AlF_6), and fluorapatite ($Ca_{10}F_2(PO_4)_6$) (NAS/NRC 1971). Whenever any of these minerals are used in industrial processes, certain amounts of them are emitted into the environment as gases or particulates. These in turn are precipitated onto ground and eventually absorbed in soils. The absorbed F may assume different forms, depending on such factors as soil pH, organic matter, clay content, and exchangeable calcium (Ca) content.

10.2.5 FOODS AND WATER

Because F is ubiquitous, virtually all foods contain trace amounts of it. Foods and beverages are therefore the most important sources of F intake. The intake from food

TABLE 10.1
Fluoride Content of Selected Foods

Food	Fluoride Content (ppm on dry basis)
Milk	0.04–0.55
Meats	0.01–7.7
Fish	0.10–24.0
Cheese	0.13–1.62
Butter	0.4–1.50
Rice and peas	10.0
Cereal and cereal products	0.10–0.20
Vegetables and tubers	0.10–2.05
Citrus fruits	0.04–0.36
Sugar	0.10–0.32
Coffee	0.2–1.6
Tea infusion	0.1–2.0
Instant (solution)	0.2

Source: Adapted from NAS/NRC Committee on Biologic Effects of Atmospheric Pollutants. *Fluorides*. National Academy of Sciences, Washington, DC, 1971, 295.

and beverages by a male residing in a fluoridated community in the United States is about 1 to 3 mg/day, while in a nonfluoridated area it is about less than 1.0 mg/day (Phipps 1996). The daily intake of F from drinking water in the United States ranges from 0.1 to 0.5 mg in nonfluoridated communities, whereas in fluoridated communities it may amount to 1–2 mg. Total F intakes in some countries are much higher than those in the United States. For instance, in the West Midlands, the longest-fluoridated region of the United Kingdom, a large number of residents were reported to take in 3 mg F or more per day (Mansfield 1998).

Plants can absorb F from soil, water, or atmosphere. F content in plants ranges from 0.1 to 10 ppm (dry basis), depending on the species. Several plant species are known as F accumulators. Examples include camellia, 620 ppm; tea leaves, 760 ppm; and elderberry, 3,600 ppm (dry basis). It is interesting to note that although tea leaves are an F accumulator, the F level in a tea beverage may be less than 0.5 mg per cup. Table 10.1 lists the F content of several varieties of foods produced in the United States.

10.3 INDUSTRIAL SOURCES OF FLUORIDE POLLUTION

10.3.1 INTRODUCTION

Anthropogenic sources of F emission include a variety of industries, such as primary aluminum production, phosphate fertilizer and elemental phosphorus plants, primary iron and steel production, and the ceramic industry (tile, brick, glass works, etc.). Combustion of fuel, especially coals and solid waste incineration, also causes F

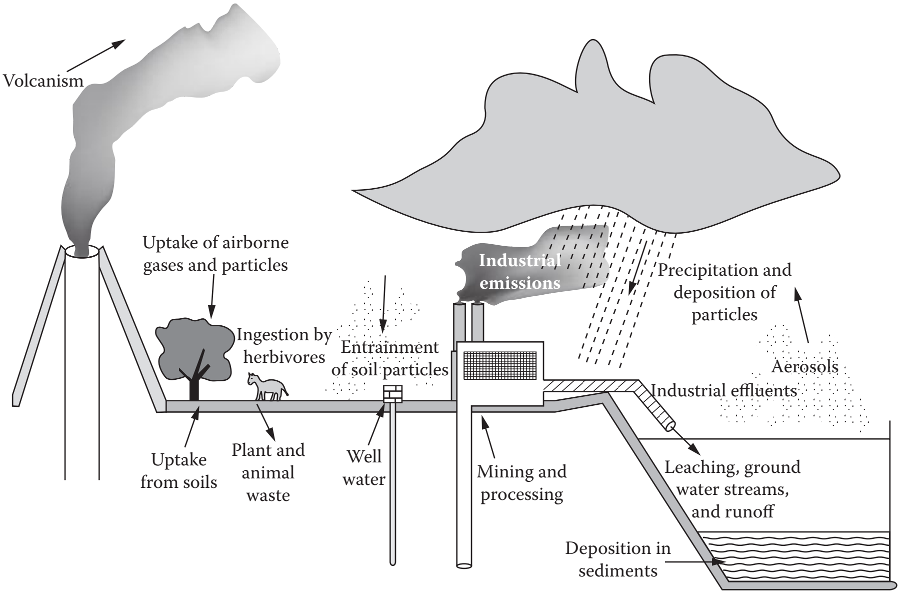

Volcanism
Uptake of airborne gases and particles
Industrial emissions
Precipitation and deposition of particles
Ingestion by herbivores
Entrainment of soil particles
Aerosols
Industrial effluents
Uptake from soils
Plant and animal waste
Well water
Mining and processing
Leaching, ground water streams, and runoff
Deposition in sediments

FIGURE 10.2 Environmental transfer of fluoride. (Adapted from NAS/NRC Committee on Biologic Effects of Atmospheric Pollutants. *Fluorides*. National Academy of Sciences, Washington, DC, 1971, 295.)

emission. The fluoride content in coal ranges from 0.001% to 0.048% in the United States (average 0.008%). The forms of F emitted from industrial processes include hydrogen fluoride (HF), fluorspar, cryolite, and silicon tetrafluoride (SiF_4). In addition to deposition into surface waters, airborne F may eventually be deposited onto the ground and taken up by soils, plants, and animals. These transfers are shown in Figure 10.2.

Several representative industrial sources of F emission into the environment are discussed in the following sections.

10.3.2 MANUFACTURE OF PHOSPHATE FERTILIZERS

The starting material for manufacture of normal superphosphate fertilizer is phosphate rock, which is mainly composed of fluorapatite. In this process, fluorapatite reacts with H_2SO_4 and water, producing $CaH_4(PO_4)_2$. The overall chemical reaction for the manufacture is shown in Equation 10.1. Since the F content of the ore is approximately 3%, a substantial quantity of HF will be produced. HF reacts with SiO_2 in fluorapatite [$Ca_{10}F_2(PO_4)_6$] to form SiF_4 (gas) (Equation 10.2).

$$Ca_{10}F_2(PO_4)_6 + 7H_2SO_4 + 3H_2O \rightarrow 3CaH_4(PO_4)_2.H_2O + 7CaSO_4 + 2HF\uparrow \quad (10.1)$$

For fluorapatite,

$$SiO_2 + 4HF \rightarrow SiF_4 + 2H_2O \quad (10.2)$$

In the aqueous scrubber, SiF_4 readily reacts with water to form fluorosilicic acid (H_2SiF_6) (Equation 10.3). Fluorosilicic acid is highly soluble in water and can be readily absorbed by plants (NAS/NRC 1971).

$$3SiF_4 + 2H_2O \rightarrow SiO_2 + 2H_2SiF_6 \tag{10.3}$$

10.3.3 MANUFACTURE OF ALUMINUM

Manufacture of aluminum is carried out almost exclusively by the Hall-Herouet process: Alumina (Al_2O_3) is dissolved in molten cryolite and reduced electrolytically. The electrolytic cell contains a carbon lining, serving as both the cathode and the container for the melt. Equation 10.4 shows the chemical reaction involved in the process:

$$Al_2O_3 + 2C \xrightarrow{\text{Catalysts}} 2Al + CO + CO_2 \tag{10.4}$$

As shown in Reaction 10.4, CO and CO_2 are the two gases emitted in the process; no emission of F-containing substances is noted. In actuality, however, a number of substances are emitted in the process. This is because several catalysts, such as CaF_2, AlF_3, and cryolite, are used in the electrolysis of alumina, and as these are heated at high temperatures, some will escape from the cells, contaminating the surrounding atmosphere. In addition to CO and CO_2 shown in Equation 10.4, several other gases, including SO_2, SiF_4, HF, COS, CS_2, helium (He), and water vapor are produced in the electrolysis cells, and these are emitted into the surrounding air. Moreover, a large number of particulates, such as Al_2O_3, carbon, cryolite, AlF_3, CaF_2, Fe_2O_3, and chiolite ($Na_5Al_3F_{14}$) are also emitted.

10.3.4 MANUFACTURE OF STEEL

In the manufacture of steel, CaF_2 is used as a flux (a substance that promotes fusion of metals) in the open hearth to increase fluidity of the slags and enhance the removal of impurities, such as phosphorus and sulfur, from the melts. Fluoride compounds emitted from this operation include HF and CaF_2 (NAS/NRC 1971).

10.3.5 COMBUSTION OF COAL

As mentioned previously, coals in the United States contain about 0.001–0.048% F, usually as fluorapatite or fluorspar. Combustion of coal in power plants therefore emits considerable amounts of F into air. During the combustion, about half of the F in coal is emitted as gaseous HF and SiF_4 and particulate matter.

With a dramatic increase in the use of coal as an energy source, atmospheric F pollution has been increasing steadily in many cities and areas in the world. This trend is particularly true in a number of less-developed countries. For example, studies showed that several cities in China, such as Beijing and Chongqing, are experiencing increasing F air pollution problems resulting mostly from combustion of coal (Feng et al. 2003; Ando et al. 2001). In Beijing, coal is the dominant energy source, accounting for nearly 80% of the total energy consumption, with the remaining 20% for heating in winter. Furthermore, the coal consumed in the city is reported to contain 163 μg F/g. This is more than double the mean value of 80 μg F/g in coals from other parts of the world (Feng et al. 2003).

10.3.6 Other Sources

Another important source of F emission in Beijing is soil dust, caused by fresh concrete used for construction work in the city. Factors such as these have contributed to the elevated F concentrations of wet depositions in the city. For example, the annual volume-weighted average concentration of soluble F of ambient aerosol is reportedly 60 μg/m^3, which is 75 times higher than the F concentration of the air samples taken in the city of Morioka in northern part of Japan, which is not known for significant F pollution problems (Ando et al. 2001).

Fluoride has also been traced to runoff from application of insecticides and weed killers. In addition to deposition into surface waters, airborne F may be deposited into surface water and onto the ground, eventually taken up by soils, plants, and animals. These environmental transfers are illustrated in Figure 10.2.

10.4 EFFECTS ON PLANTS

Compared with other air pollutants, fluoride and its compounds may cause higher toxicity in plants. In fact, HF is considered the most phytotoxic air pollutant. Fluoride can cause injury to susceptible plants at concentrations below 1 ppb (0.8 μg/m^3) for exposure periods of 7 days or less (Weinstein 1983; Madkour and Weinstein 1987; Giannini et al. 1985). The high toxicity of fluorides is mainly due to their rapid absorption and inherent toxicity of F as an element. Plants being exposed to F can result in marked increases in foliage F levels. The extent of increases depends on factors such as duration of exposure, atmospheric F levels, and species or variety of plants. Fluoride-induced effects in plants may be viewed based on four levels of biologic organization: cellular levels, tissue or organ, organism, and ecosystem (Table 10.2) (NAS/NRC 1971).

Fluoride accumulates in plant leaves mainly as a result of diffusion through the stomata from the atmosphere or through absorption from soil by the root. In contrast to other major air pollutants such as SO_2, NO_2, and O_3 discussed in Chapter 8, F accumulates in the foliage of plants, which serves as a vehicle for its transfer to herbivores with the potential for inducing dental and skeletal fluorosis.

Fluoride induces both structural and functional changes in plant cells. Changes occur in cellular and subcellular membranes with subsequent injuries. Although plants differ widely in their susceptibility to F injury, accumulation of high levels of F in leaves normally leads to chlorosis and necrosis (Figure 10.3). Chlorosis is associated with lowered chlorophyll content in the leaf, thus lowering photosynthesis. Similarly, the destruction of part of the leaf resulting from necrosis will lead to a comparable reduction in photosynthesis (McLaughlin et al. 1982). Both chlorosis and necrosis lead to reduced plant growth and yield. Tree death could result when the injuries are severe. Contrary to that of NO_2 or SO_2, F-induced damage occurs in leaf tips and margins of many plant species.

TABLE 10.2
Nature of Fluoride-Induced Effects in Plants at Four Levels of Biologic Organization

Cellular	Tissue or Organ	Organism	Ecosystem
Effects on enzymes and metabolites	Decreased assimilation Altered respiration		Increased fluoride in ecosystem
Modification of cell organelles and metabolism	Altered growth and development	Modified growth	Increased fluoride burden of animals
Pathway disruption	Chlorotic lesions	Reduced reproduction	Fluorosis in animals
Cellular modification	Necrotic lesions	Decreased fitness for environment	
Disruption and death of cell	Death or abscission of leaf	Death of plants	Desolation

Source: Adapted from NAS/NRC Committee on Biologic Effects of Atmospheric Pollutants. *Fluorides*. National Academy of Sciences, Washington, DC, 1971, 79.

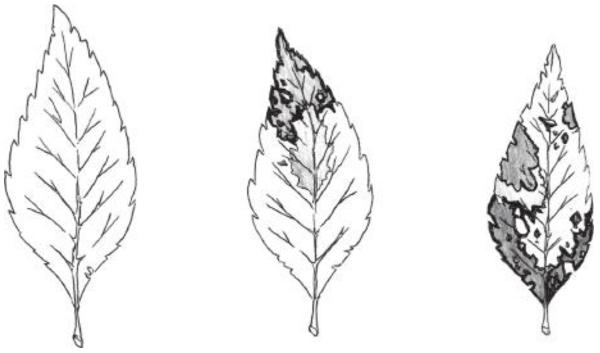

(a) (b) (c)

FIGURE 10.3 Plant leaves exhibiting chlorotic and necrotic lesions. (a) Normal leaf; (b) chlorotic leaf; (c) necrotic leaf. (From Yu, M.H. 2003. Unpublished data.)

Plants exposed to various concentrations of F in the laboratory generally exhibit concentration-dependent impairment of growth. For example, mung bean seedlings exposed to 0, 0.1, 1.0, and 5.0 m*M* F (as NaF) for 24, 48, and 72 hours showed inhibition of germination. Seedlings treated with 1.0 and 5.0 m*M* F for 72 hours showed 33% and 73% decreases in radicle length, respectively (Table 10.3). A similar observation was made for rice (*Oryza sativa*) by Gupta et al. (Gupta 2009). These researchers exposed rice seeds to 0, 10, 20, and 30 mg NaF/L for 15 days and found that the seeds treated with 0 and 10 mg NaF/L showed 100% germination, but in those treated with 20 and 30 mg NaF/L, germination was reduced to 92% and 96%,

TABLE 10.3
Effect of Fluoride on Fresh Weight and Root Elongation in Mung Bean Seedlings Exposed to NaF

NaF (m*M*)	Radicle Weight (mg/seed)	%	Radicle Length (mm)	%
0	139 ± 8.2	100	77 ± 10.9	100
0.1	125 ± 11.2	90	73 ± 15.1	95
1.0	117 ± 16.1*	84	52 ± 8.2***	67
5.0	35 ± 5.7***	25	21 ± 4.6***	27

Note: Values are mean ± SD ($N = 15$).

* $p < .05$; ***$p < .001$.

Source: Yu, M.H. 2004. Unpublished data.

respectively. In addition, seeds exposed to 30 mg NaF/L resulted in decreases in root length, shoot length, and dry weight by 50%, 27%, and 29%, respectively.

The inhibitory effects of F on plants are often found in field studies, as shown in Case Study 10.1.

Case Study 10.1

In 1979, researchers in Taiwan observed a previously unknown foliage disease of rice plants grown in the northern part of Taiwan. Leaves of the plants grown in an area adjacent to ceramic and brick industrial facilities manifested acute symptoms of chlorosis and tip necrosis. Studies done in 1983 showed ambient F concentration in the area to range from 0.4 to 15 µg/kg (average 4.5 µg/kg). Analysis of the leaf F contents in rice plants grown in the area revealed marked increases, and the levels were correlated with increasing severity of the leaf injury. The severely injured leaves contained 80 times as much F as the healthy leaves did. Subsequently, laboratory experiments involving fumigation of rice seedlings with HF were conducted. The results showed leaf symptoms that were similar to those observed in the field. These observations suggested to the researchers that F emitted from the ceramic and brick factories was the cause of the observed "new rice disease" (Sun and Su 1985).

10.5 EFFECTS ON ANIMALS

10.5.1 INTRODUCTION

Animals normally ingest small amounts of F in their rations without observable adverse effects, but excessive intake can be detrimental. Common sources of excessive F intake by animals include forages subjected to airborne contamination or grown in soils containing high F levels and water or feed supplements containing high levels of F. The effect of F on domestic animals may be acute or chronic, depending on the levels to which animals were exposed.

10.5.2 Acute Effects

Fluoride has caused detrimental effects on livestock in the United States and several other industrialized countries. The sources of F pollution are limited mostly to phosphate fertilizer manufacturing, aluminum production, fluorohydrocarbons, and heavy metal production. A safe level of soluble F in animal rations ranges from 30 to 50 mg/kg for cattle and from 70 to 100 mg/kg for sheep and swine. F poisoning may cause physiological effects, such as gastroenteritis, muscular weakness, pulmonary congestion, nausea, vomiting, diarrhea, chronic convulsions, necrosis of mucosa of the digestive tract, anorexia, cramping, collapse, and respiratory and cardiac failure, possibly leading to death.

10.5.3 Chronic Effects

The two most conspicuous and thoroughly studied manifestations of chronic F poisoning are dental and skeletal fluorosis. Once absorbed into the animal body, F has a great affinity for developing and mineralizing teeth. This affinity can either enhance tooth development or induce dental lesions, depending on the amounts of fluoride ingested. Dental fluorosis is the first sign of chronic F toxicity. It is exhibited by white, yellow, brown, and black discoloration of tooth enamel, either in spots or in horizontal streaks. An affected tooth is also subject to more rapid wear and erosion of the enamel from the dentin. It is noteworthy that dental lesions will not be seen in animals brought into endemic fluorosis areas after their permanent teeth have erupted (Shupe and Olson 1983).

Studies indicated a widespread chronic effect of environmental F on wildlife. F contamination of vegetation arises from various industrial activities, noted previously. Studies conducted by European scientists showed a large number of deer in several European countries suffering from both dental and skeletal fluorosis (Kierdorf et al. 1996; Vikoren et al. 1996). However, since about 1980, much improvement has been made in controlling F emission, as evidenced by comparative field studies showing that F contamination of vegetation has decreased significantly in recent years. Concomitantly, decreases were observed in F levels in dental and skeletal samples of wild animals.

Studies of deer population affected by F are relatively limited in the United States. There is no active research in this area, presumably because atmospheric F pollution is limited to local areas and is not considered a serious environmental problem. Nevertheless, studies have demonstrated increased bone F levels in other animals found around F-emitting industrial facilities. Furthermore, it is often found that F levels are inversely related to the distance between the industrial facilities and the site of animal collection. For example, the mean F levels in bones of deer mice collected from various sites from around an aluminum plant in North America were found to be 1, 724 ± 142 mg/kg and 237 ± 89 mg/kg dry weight for animals collected at 2 km and 32 km from the plant, respectively (Figure 10.4) (M.H. Yu, unpublished data, 2003).

The impact of airborne F on wildlife is also demonstrated in the teeth of black-tailed deer. Figure 10.5 shows a comparison between a normal tooth wear pattern

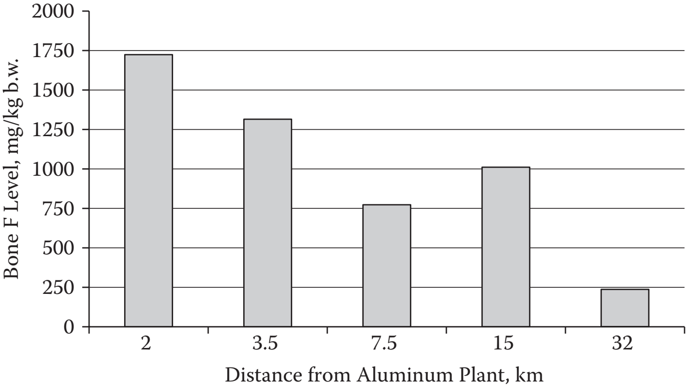
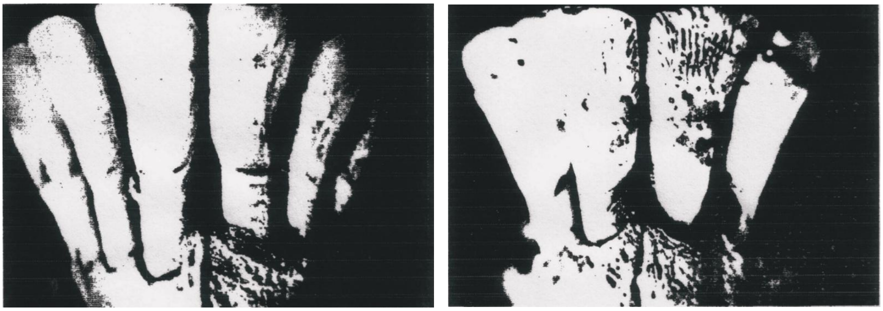


FIGURE 10.4 Bone fluoride levels in deer mice from an area adjacent to an aluminum plant.



(a) (b)

FIGURE 10.5 (a) Normal tooth wear patterns of a black-tailed deer (male, ca. 2.5 years old) killed in an area with no industrial facilities. (b) Dental disfigurement and abnormal tooth wear patterns in a black-tailed deer (female, ca. 1.5 years old) killed in an area adjacent to an aluminum plant.

of a male black-tailed deer (deer A) and an abnormal tooth wear pattern of a female black-tailed deer (deer B). Deer A was killed on a road in an area with no industrial facilities, whereas deer B was killed on a road adjacent to an aluminum manufacturing plant. Analysis of the F content in bone samples from these two animals showed that the F levels of the bone from deer B was 15 to 20 times higher than those of the bone from deer A (Newman and Yu 1976).

In addition to inducing tooth mottling, F can cause skeletal fluorosis. Skeletal fluorosis causes bones to lose their normal, hard, smooth luster and appear rough, porous, and chalky white. A generalized hyperostosis (excessive formation of bone tissue, especially in the skull) and, in some cases, exostotic lesions of the otherwise-smooth long bones may be observed (exostosis is a spur or bony outgrowth from a bone) (Figure 10.6). Lameness or stiffness is an intermittent sign of F toxicity. The clinical basis for the lameness is not well understood.

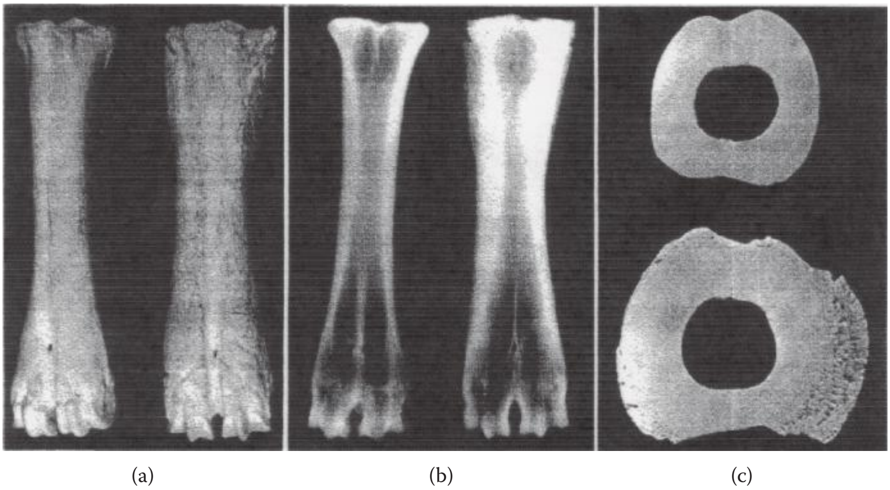


FIGURE 10.6 Bones from dairy cows with skeletal fluorosis. (a) Metatarsal bone from dairy cow fed 12 ppm fluoride from 3–4 months to 7.5 years of age. The bone is normal. Right: Metatarsal bone from a dairy cow fed 93 ppm fluoride for the same period. The bone shows marked periosteal hyperostosis with a roughened surface. (b) Radiographic comparison of bones in (a). (c) Upper: Cross section of a metatarsal bone from a dairy cow fed 12 ppm fluoride from 3–4 months to 7.5 years of age. The bone is normal. Lower: Cross section of a metatarsal bone from cow ingesting 93 ppm fluoride for the same period. The bone shows definite osteofluorosis. (Adapted from Greenwood, D.A., J.L. Shupe, G.E. Stoddard, L.E. Harris, H.M. Nielsen, and L.E. Olson. *Fluorosis in cattle.* Special Report 17. Agricultural Experiment Station, Utah State University, Logan, Utah, 1964, 1–36.)

Impaired appetite is normally observed, which may result in decreased weight gain, cachexia, and lower milk yield. Decrease in milk production may be secondary to appetite impairment or other responses. Evidence that animals may be suffering chronic F effect may be obtained from chemical analysis of the feed and elevated levels of F in urine and body tissues (Parker et al. 1979). Affected animals may exhibit increased susceptibility to other environmental stresses and decrease in longevity.

A number of factors influence the manifestation of dental and skeletal fluorosis. They include

- amount and bioavailability of F ingested
- duration of ingestion
  - species of animals involved (Table 10.4)
  - age at time of F ingestion
  - mode of F exposure (e.g., continuous or intermittent)
  - individual biologic response
  - presence of synergistic or antagonistic substances
  - nutritional and general health status of animals
  - presence of other stress factors, such as those caused by poor management

TABLE 10.4
Fluoride Tolerances (ppm as NaF) in Livestock Diets

	Breeding or Lactating Animals	Finishing Animals
Dairy and beef heifers	30	100
Dairy cows	30–50	100
Beef cows	40–50	100
Sheep	70–100	160
Horses	60	—
Swine	70–100	—
Turkeys	300–400	100
Chickens	150	—

Source: Adapted from NAS/NRC Committee on Biologic Effects of Atmospheric Pollutants. *Fluorides*. National Academy of Sciences, Washington, DC, 1971, 152.

10.6 EFFECTS ON HUMANS

10.6.1 Daily Intake of F

Daily intake of F by individuals in the United States is about 0.2 to 0.3 mg from food, 0.1 to 0.5 mg from water (1 to 2 mg if water is fluoridated), and varying quantities from beverages (F content of wine is 0 to 6.3 ppm; beer, 0.15 to 0.86 ppm; milk, 0.04 to 0.55 ppm). The amount of F inhaled from air is about 0.05 mg/day.

10.6.2 Absorption

Absorption of F from the gastrointestinal tract occurs through a passive process; it does not involve active transport (NAS/NRC 1971). The absorption is rapid and probably occurs in the lumen. The rate of absorption depends on the F compounds involved, for example, 97% of NaF, 87% of $Ca_{10}F_2(PO_4)_6$, 77% of Na_3AlF_6, and 62% of CaF_2 are absorbed. About 50% of the absorbed F is excreted by the kidneys, while the remainder is stored, primarily in calcified tissues. No significant F accumulation occurs in soft tissues.

Bone has a great affinity for F and incorporates it into hydroxyapatite ($Ca_{10}[OH]_2[PO_4]_6$), forming fluorapatite ($Ca_{10}F_2[PO_4]_6$). Even at low levels of F intake, appreciable levels of F will, in time, accumulate in calcified tissues. The effectiveness of low levels of F intake in reducing dental caries in humans and rats and some other species of animals has been reported. In the human population, a water supply containing 1 ppm F is reported to reduce more than 50% of dental caries incidence in individuals who consume F from infancy. Fluoride is incorporated into tooth mineral as fluorapatite at the time of calcification.

10.6.3 ACUTE EFFECTS

Exposure to high levels of F results in varying degrees of injuries (see, for example, Case Study 10.2). The lethal dose of inorganic F has been estimated to be in the range of 2.5 to 5 g for a 70-kg male, or approximately 50 mg/kg, a dose similar to the LD_{50} (amount sufficient to kill 50%) for several animal species. The cause of death has been suggested to be related to the prompt binding of F with serum calcium and magnesium (Mg). Clinical symptoms include excessive salivation, perspiration, vomiting, painful spasms of limbs, stiffness, nausea, chronic convulsion, necrosis of the mucosa of the digestive tract, and heart failure.

Case Study 10.2

A fluoride overfeed occurred in 2002 at a well site near an elementary school in Portage, Michigan. The incident resulted in a high F concentration in the drinking water (92 mg/L) at the school. Several students who drank water from the school fountain reportedly suffered nausea and vomiting. Toxicological studies were conducted to assess the risk. Based on the symptoms experienced by the students, it was concluded that the F had irritated the stomach, causing the observed symptoms, but that no appreciable long-term adverse health effects would occur (Sidhu and Kimmer 2002).

10.6.4 CHRONIC EFFECTS

Fluoride accumulates in the skeleton during prolonged, high-level exposures. Radiological evidence of hypermineralization (osteofluorosis) is shown when bone F concentrations reach about 5,000 ppm (Hodge and Smith 1965). Coupled with other environmental factors, such as poor nutrition and health status, patients may suffer severe skeletal dysfunction. In addition, vomiting and neurological complaints have been observed in some patients. Increased levels of serum and urinary F usually occur. Fluoride exposure leads to cell damage and induces necrosis. Eventually, giving F orally produces massive impairment in the functions of vital organs.

In some parts of the world, such as India, Mexico, and China, the water supplies in many villages (usually from wells) contain high levels of F, in some cases higher than 20 ppm. As a result, osteofluorosis among the residents is common. Published reports indicate that China may have about 20 million people afflicted by chronic F poisoning (Yu and Tsunoda 1988). As mentioned previously, the dramatic increase in the use of coal as an energy source has resulted in many parts of China with rising emissions of F into the environment. Research conducted by Ando et al. (2001) showed high incidences of dental and skeletal fluorosis in some rural areas in that country.

Wang et al. (2009) studied the F level in several environmental samples collected from two villages, A and B, in Shaanxi Province in China. F levels in samples from village A were high, whereas those from village B were low (control). They found that in village A, the F levels in the samples were 1,757 mg/kg in coal, 0.007 mg/L in drinking water, 1.47 mg/kg in soil, 4.78 mg/kg in corn, and 31.79 mg/kg in chili, whereas the F levels in village B were120 mg/kg in coal, 0.008 mg/L in drinking water, 0.64 mg/kg in soil, 2,69 mg/kg in corn, and 7.98 mg/kg in chili. The incidence of skeletal fluorosis in village A was 6% for male subjects and 4% for female subjects, whereas the incidence in village B was 0% for both male and female subjects.

India is known to be one of the 23 nations in the world where health problems occur due to excess intake of F in drinking water (>1.5 mg/L). For example, Hussain et al. (2009) reported that the F concentrations of 73% of the 1,030 villages in the district of Bhilwara in Central Rajasthan in India were above 1.0 mg/L (range 0.2 to 13.0 mg/L), and that the levels of 5.8% of the villages studied were above 5.0 mg/L. A large number of the residents showed dental fluorosis and skeletal fluorosis.

Dental fluorosis is the first sign of chronic F toxicity. It is manifested by white, yellow, brown, and black discoloration of tooth enamel, either in spots or in horizontal streaks. One of the earliest symptoms of dental fluorosis is mottled enamel.

Skeletal fluorosis refers to accumulation of F in skeletal tissues and is associated with pathological bone formation. It is one of the most severe effects of F on humans and is caused by intake of elevated levels of F over a long period. In the F-afflicted areas studied by Ando et al. (2001), combustion of coal and coal bricks was found to be the primary source of gaseous and aerosol F in the human environment. Airborne F from the combustion of coal was found to pollute extensively both the living environment and the food, such as corn, chilies, and potatoes, consumed by the residents.

Several groups of researchers have reported reproductive effects of F in humans (Narayana and Chinoy 1994; Freni 1994). More recently, Ortiz-Pérez et al. (2003) studied the reproductive parameters in Mexican residents. Two groups of male residents were identified: the high-fluoride-exposed group (HFEG), those exposed to F at 3 to 27 mg/day, and the low-fluoride-exposed group (LFHG), exposed to 2 to 13 mg/day. Increased urinary F levels (3.2 mg F/g creatinine vs. 1.6 mg F/g creatinine) were found in the HFEG compared with the LFEG. Levels of reproductive hormones were also measured; the HFEG showed higher follicle-stimulating hormone but lower inhibin B, prolactin, and free testosterone serum concentrations than the LFEG, while no differences were found for total testosterone, estradiol, or lutenizing hormone between the two groups (Ortiz-Pérez et al. 2003).

10.7 BIOCHEMICAL EFFECT OF FLUORIDE

10.7.1 In Plants

Fluoride is widely known as a metabolic inhibitor. In plants, F affects many biological processes, including glycolysis, TCA (tricarboxylic acid) cycle reactions, photosynthesis (Weinstein 1983; Madkour and Weinstein 1987), protein synthesis, lipid metabolism, and others. Much of the action of F on these processes can be attributed to F inhibition of enzymes. Enzymes that are inhibited by F include enolase, phosphoglucomutase, phosphatase, hexokinase, PEP (phosphoenol puruvate) carboxylase, pyruvate kinase, succinic dehydrogenase, malic dehydrogenase, pyrophosphatase, phytase, nitrate reductase, mitochondrial ATPase (adenosine triphosphatase), and urease (Miller et al. 1983).

Figure 10.7 gives a diagram showing the physiological features of a germinating mung bean seedling. Inhibition of amylase (Figures 10.7 and 10.8) (Yu et al. 1988)

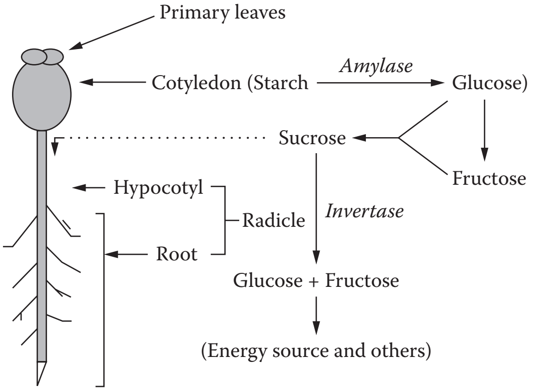

Primary leaves
Cotyledon (Starch
Amylase
Glucose)
Sucrose
Fructose
Hypocotyl
Radicle
Root
Invertase
Glucose + Fructose
(Energy source and others)

FIGURE 10.7 A diagram showing the physiological feature of germinating mung bean seedling.

and invertase (Yu 1997) (Figure 10.7) in vivo was observed in germinating mung bean seedlings exposed to NaF at 1 m*M* and above. Fluoride-induced inhibition of amylase and invertase appears to involve the removal of cofactor Ca^{2+} by F. In a separate study, the inhibition of lipase was also shown in the seedlings (Yu et al. 1987).

The inhibition of plant enzymes such as these is often reflected by compositional changes in tissues. For example, Yang and Miller (1963) showed that the sucrose content in soybean leaves exposed to 30 ppb of HF was increased, whereas the levels of both glucose and fructose were elevated. They also observed marked increases in several organic acids, including malic, malonic, succinic, and citric acids.

Similarly, the inhibition of amylase in mung bean seedlings by F resulted in lowered starch breakdown in cotyledon and thus a reduced glucose level (Figure 10.8) (Yu et al. 1988).

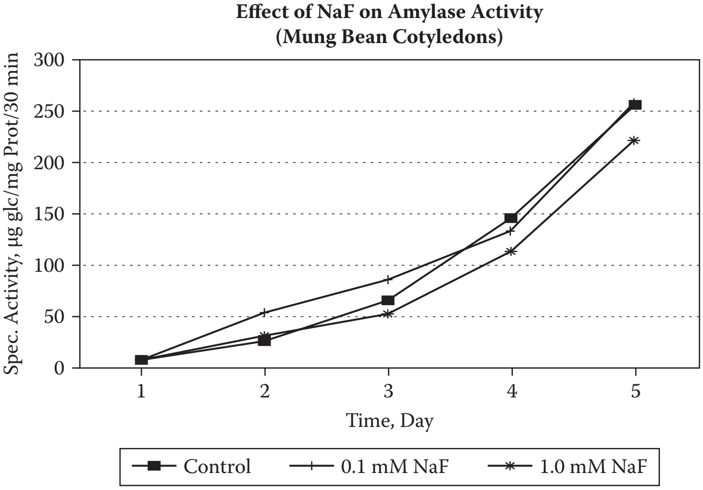


FIGURE 10.8 Inhibition of mung bean amylase activity by NaF.

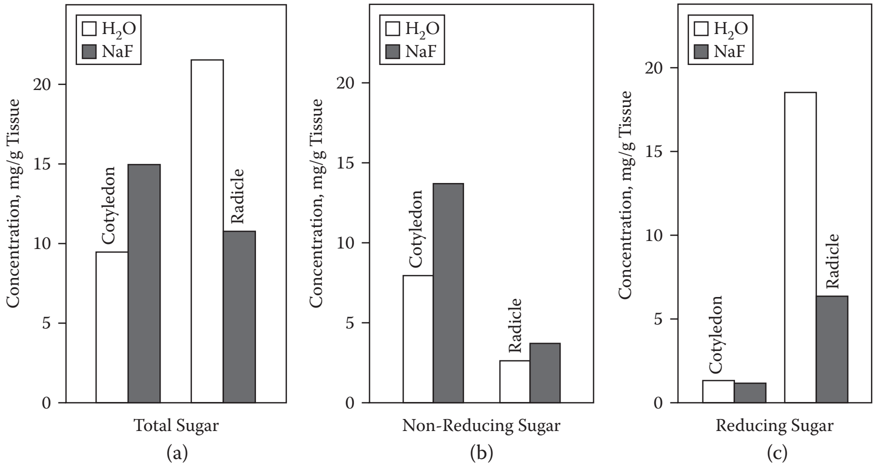


FIGURE 10.9 Effect of NaF on sugar content in germinating mung bean seedling. (a) total sugar; (b) nonreducing sugar; (c) reducing sugar.

Furthermore, the inhibition of invertase in the radicle leads to impaired breakdown of sucrose to glucose and fructose (Yu 1997). These results are evident from the accumulation of nonreducing sugar (in this case sucrose) in the cotyledon and radicle (Yu 1996). On the other hand, marked decreases in reducing sugar (glucose and fructose), particularly in radicle, are shown (Figures 10.9a–10.9c).

As noted in Chapter 6, superoxide dismutase (SOD) is an important antioxidant enzyme. Field and laboratory studies have shown that SOD activities in different plant tissues exposed to F were either enhanced or lowered. For instance, mung bean seedlings exposed to 0.1 m*M* NaF showed an enhanced SOD activity, whereas exposure to 0.2 m*M* NaF and above resulted in depressed SOD activity (Figure 10.10) (Wilde and Yu 1998).

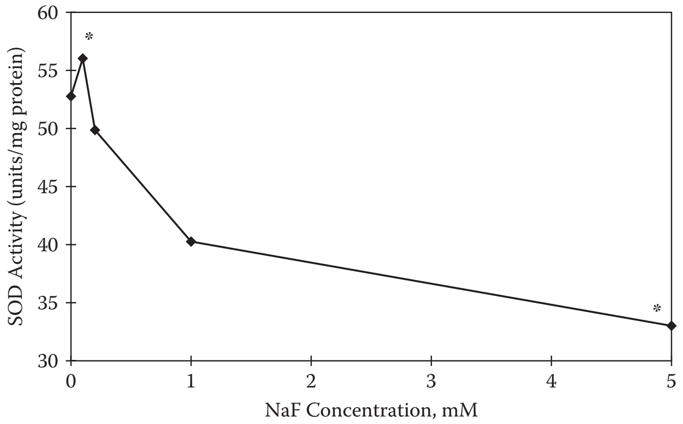
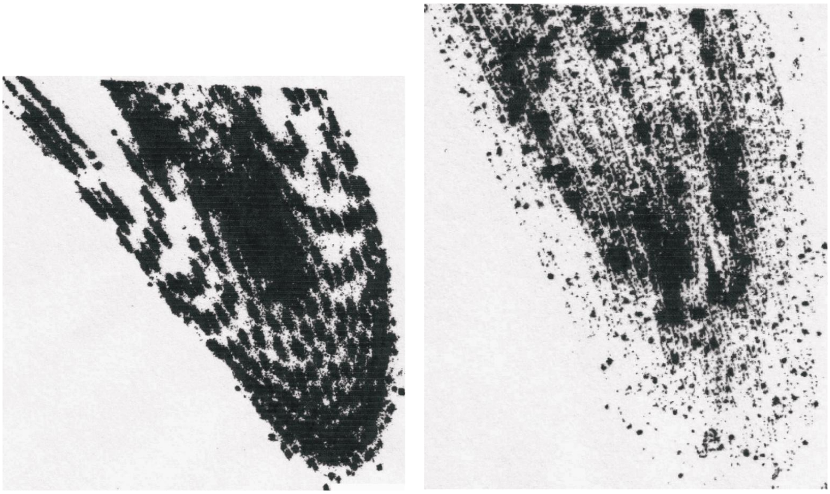


FIGURE 10.10 Effect of NaF on SOD activity in mung bean seedlings.



(a) (b)

FIGURE 10.11 Autoradiographs showing [2-^{14}C]thymidine incorporation in radicle of mung bean seedling treated with (a) water or (b) 1 m*M* NaF for 12 hours. (Black grains show the sites of [2-^{14}C]thymidine incorporation into DNA in the tissue.) (From Narita, Nakamura, Shigematsu, and Yu. *Fluoride*, 29, 72. 1996.)

In a separate study, Narita et al. (1996) showed that F inhibited [2-^{14}C]thymidine incorporation into DNA in mung bean seedlings exposed to 1 m*M* NaF for 24 hours and above (Figure 10.11). The inhibition suggests a concomitant influence on protein synthesis.

While it is clear that the action of F on metabolism is complex and involves a variety of enzymes, the mode of action of fluoride ion (F^-) on these enzymes is not so clear. Nevertheless, the principal mechanisms that have been suggested include

- formation of complexes with metalloenzymes
- removal of a metal cofactor, such as calcium or magnesium, from an enzyme system
- binding to the free enzyme or to the enzyme substrate complex (Giannini et al. 1985)
- disruption of hydrogen bonds on protein molecules (Yu 1997)

Because hydrogen bonding is important in the maintenance of the tertiary structure of protein molecules, disruption of an enzyme protein by F would lead to enzyme inhibition.

10.7.2 IN ANIMALS AND HUMANS

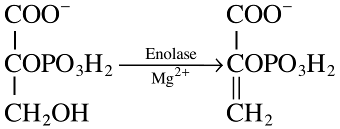
Fluoride inhibits the metabolism of carbohydrates, lipids, and proteins. In animals and humans, a large number of enzymes are inhibited by F, including enolase, ATPase (adenosine triphosphate [ATP] → adenosine diphosphate [ADP] + Pi [inorganic phosphate]), lipase, and cholinesterase. Inhibition of glycolysis, due in part to decreased enolase activity, may be responsible for the hyperglycemia observed in laboratory animals exposed to F.

F stimulates adenylcyclase activity in all tissues so far examined (adenylcyclase catalyzes the formation of cyclic adenosine monophosphate [cAMP] from ATP). F also affects functions controlled by calcium in humans, as it does in plants. These functions include blood clotting, membrane permeability, and cholinesterase activity. F inhibition of reactions involving calcium is generally attributed to the formation of CaF_2, as shown in Reactions 10.5 and 10.6:

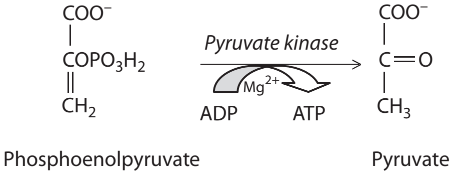
$$[\text{Protein-Ca}] \rightleftharpoons \text{Protein} + \text{Ca}^{2+} \tag{10.5}$$

$$\text{Ca}^{2+} + 2\text{F}^- \rightleftharpoons [\text{CaF}]^+ + \text{F}^- \rightleftharpoons \text{CaF}_2 \tag{10.6}$$

Enzyme systems requiring magnesium are also mediated by F. For example, F has been shown to inhibit enolase, a magnesium-requiring enzyme responsible for the conversion of 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway (Reaction 10.7). According to some researchers, the inhibition results from the formation of a magnesium-fluorophosphate complex, thus essentially making magnesium unavailable for the enzyme. Magnesium is also required in the next step, where phosphoenolpyruvate is converted to pyruvate (Reaction 10.8) to complete the glycolysis pathway (Park et al. 1999). As shown in Reaction 10.8, this step yields energy in the form of ATP from ADP.



$$\begin{array}{l}\text{COO}^-\\ |\\ \text{COPO}_3\text{H}_2\\ |\\ \text{CH}_2\text{OH}\end{array} \xrightarrow[\text{Mg}^{2+}]{\text{Enolase}} \begin{array}{l}\text{COO}^-\\ |\\ \text{COPO}_3\text{H}_2\\ \|\\ \text{CH}_2\end{array} \tag{10.7}$$



(10.8)

The inhibition of myosin ATPase by F is another example of F interacting with magnesium. Energy transduction in myosin converts the chemical energy released by ATP into mechanical work at the site of force generation. Myosin is a fibrous globulin that interacts with actin (a protein in muscle that is active in muscular contraction) and ATP, with resulting enzymatic hydrolysis of ATP to ADP and Pi:

$$\text{ATP} \xrightarrow{\text{ATPase}} \text{ADP} + \text{Pi} \tag{10.9}$$

During hydrolysis of ATP, myosin subfragment 1 (S1) requires the presence of Mg^{2+} ion to stabilize the nucleotide or nucleotide analog in the active site of S1. In the presence of F, Mg^{2+} and MgADP form a complex MgADP-MgFx that traps the active site of S1 and inhibits myosin ATPase (Park et al. 1999).

As noted, F is shown to inhibit protective enzymes, such as SOD, glutathione peroxidase (GSHPx), and catalase in various human tissues. Inhibition of one or more of these enzymes may allow a free-radical-induced reaction to occur, leading to cellular and tissue damages.

In a 2006 review by the U.S. National Research Council (NRC), F (as F^- ions) is described as an "endocrine disruptor." According to NRC, F is an endocrine disruptor in the broad sense, altering normal endocrine function or response, although probably not in the sense of mimicking a normal hormone. Further action is necessary to explore the possibility to characterize the direct and indirect mechanisms of fluoride's action (National Research Council 2006).

10.8 NUTRITION AND FLUORIDE TOXICITY

Several nutrients have been shown to alleviate injuries caused by exposure to F. The nutrients studied so far include proteins, calcium, and vitamins C (ascorbic acid), D, and E. Glutathione (GSH), which is not a nutrient but a well-known antioxidant, has also been studied. The adverse effects of F are often mitigated by administration of one or more of these substances.

A large number of studies have indicated the relationship between vitamin C and F exposure in animals. In one of these studies, growing chicks (*Gallus domesticus*) were fed a diet supplemented with 150 ppm of F as NaF (Yu and Driver 1978). At the end of 4 weeks, no differences in body weight were observed; however, F-treated chicks showed a marked decrease in ascorbic acid levels in the heart, spleen, brain, gizzard, and pancreas, while the levels were increased in the lungs and kidneys. In a separate study, growing cockerels were given a diet supplemented with 150, 300, or 500 ppm F for 4 or 8 weeks and then subjected to analysis of tissue levels of ascorbic acid and dehydroascorbic acid (DHA, the oxidized form of ascorbic acid). Results showed marked decline of ascorbic acid in adrenal glands and kidneys in the F-treated cockerels. Furthermore, the levels of DHA in the kidneys of the F-exposed cockerels increased more than 100% compared with the control levels (Yu and Driver 1982).

In laboratory mice, both protein and vitamin C were shown to lower F accumulation in bone. For example, mice fed a low-protein diet (containing 4% protein) supplemented with 150 ppm NaF deposited five times more F in the tibia than did control mice fed a regular diet (containing 27% protein) and exposed to the same level of NaF. Furthermore, supplemental vitamin C greatly reduced F deposition in the bone. (It should be noted that mice also produce vitamin C.) (Yu and Hwang 1986.)

Fluoride treatment has been shown to induce embryotoxicity in pregnant rats (Verma and Sherlin 2001). Oral administration of NaF (40 mg/kg body weight) to pregnant rats from day 6 of gestation to day 19 caused decreased body weight, feed consumption, absolute uterine weight, and number of implantations, compared with the control. A higher incidence of skeletal and visceral abnormalities (subcutaneous hemorrhage) was observed in the fetuses of F-treated pregnant rats. Oral administration of vitamin C (50 mg/kg body weight) with NaF significantly reduced the severity and incidence of F-induced embryotoxicity in the rats.

Exposure of male mice to NaF (10 mg/kg body weight) for 30 days showed a marked decrease in cauda epididymal sperm count, motility, and viability, resulting in significant reduction in fertility rate (Chinoy and Sharma 1998). Withdrawal of NaF treatment for 30 days produced incomplete recovery. However, when vitamin E or vitamin D was supplemented during the withdrawal period, the toxic effect of NaF was significantly alleviated, as the treated mice restored their reproductive functions and fertility. In addition, it was found that a combined administration of vitamins D and E was generally more effective than either vitamin D or E administered alone.

According to the NRC, F has the potential of disrupting the function of many tissues that require iodine (I_2) or iodide (I^-) (National Research Council 2006). A number of researchers have implicated the links between iodine deficiency and fibrocystic breast disease. According to Clinch (2009), iodine appears to increase urinary excretion of F.

REVIEW QUESTIONS

1. Why is fluoride the most phytotoxic of the major air pollutants?
2. What are the most important fluoride-containing minerals?
3. List three industrial operations that result in atmospheric emission of fluoride.
4. What is the reason for fluoride emission in the aluminum manufacturing process?
5. What are the characteristic symptoms of leaf injury induced by exposure to high levels of fluoride?
6. How does fluoride affect seed germination?
7. In field studies, what can generally be observed concerning the relationship between bone fluoride level of small animals and the distance of the collection site from a nearby aluminum plant?
8. Explain the dental lesions manifested by animals and humans suffering chronic fluoride poisoning.
9. Of the following fluoride-containing compounds, which one has the highest rate of absorption? Which has the lowest rate of absorption? (a) NaF, (b) cryolite, (c) CaF_2, (d) fluorapatite
10. What is the suggested mode of action for fluoride?
11. How is fluoride toxicity related to tissue calcium levels?
12. What effect does vitamin C have on fluoride toxicity in animals and humans?
13. How does fluoride affect enolase?
14. How is fluoride related to the protective enzymes?
15. Which nutrients have been shown to be capable of alleviating fluoride toxicity?

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11 Volatile Organic Compounds

11.1 INTRODUCTION

Volatile organic compounds (VOCs) are those organic compounds with a boiling point range of 50–100 to 240–260°C (World Health Organization [WHO] 1989). They include a large number of major air pollutants emitted from both industrial and nonindustrial facilities. Chemically, VOCs include both aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, some alcohols, esters, and aldehydes. Table 11.1 shows several examples of this group of compounds. In this chapter, the sources, characteristics, and health effects of some VOCs are discussed.

11.2 SOURCES

Both natural and anthropogenic sources contribute to VOC emissions. Natural sources of VOCs include petroleum, forest fires, and the transformation of biogenic precursors. Main anthropogenic sources include high-temperature combustion of fuels, emissions from crude and refined oil, municipal incineration, burning of crops before or after harvesting as an agricultural practice, emissions from power boats, and others. In terms of the quantities of anthropogenic material emitted in the United States during 1998, five major air pollutants—carbon monoxide (CO), sulfur oxides (SO_x), nitrogen oxides (NO_x), VOCs, and particulate matter (PM)—accounted for 98% of total pollution, and VOC emission alone contributed 14%. Following the passage of the Clean Air Act of 1970, VOC emissions, like most other emissions, decreased significantly.

However, a survey by the EPA showed indoor pollution as an important source of VOCs. In a survey involving 10 buildings, more than 500 VOCs were detected, although most were detected only once. The study showed that a variety of VOCs originated from common items, such as building materials, cleaning solvents, furnishings, and pesticides. Because indoor concentrations for all compounds except benzene exceeded outdoor levels, it was concluded that indoor sources contributed to the observed results. The importance of indoor VOC pollution in health has been recognized in World Health Organization (WHO) publications (1989).

11.3 PETROLEUM HYDROCARBONS

Petroleum is a complex mixture of hydrocarbons, with a characteristic chemical composition and specific physical properties, depending on the geological and geographical origin of the crude oil and the nature of the cracking process used during refining. Petroleum hydrocarbon components are divided into three major

TABLE 11.1
Examples of Volatile Organic Compounds

Group	Examples
Aliphatics	Pentene, hexane, heptane, cyclohexane, octane, nonane eicosane, dodecane, 2,4-dimethylhexane
Aromatic hydrocarbons	Benzene, diethylbenzene, trimethylbenzenes, dimethyl-ethylbenzene, toluene, xylenes, naphthalene, styrene
Halogenated hydrocarbons	Chloroform, dichloromethane, trichloroethylene, tetrachloroethylene, dichlorobenzene
Alcohols	2-Butylalcohol, 1-dodecanol
Aldehydes	Decanal, nonanal
Esters	Ethyl acetate, 1-hexyl butanoate

TABLE 11.2
Comparison between Aliphatics and PAH

Characteristics	Aliphatics	PAH
Rate of degradation	Faster	Slower
Persistence in tissue	Shorter	Longer
Toxicity	Less toxic	More toxic (some are carcinogenic)

classes related to their chemical structure: the alkanes, the alkenes, and the aromatics (Boesch 1974). These compounds enter the environment as a result of stationary and mobile sources and comprise a significant portion of the contaminant mixture found in ground- and surface waters, coastal areas, and the global atmosphere (Berry and Bammer 1977; Singh 1992). The drilling, removal, processing, transportation, storage, and use of petroleum hydrocarbons involve several operations, during which losses of material, chemical conversions, and discharges of wastes can occur. Total global emissions and discharges of petroleum have been estimated at about 90 million tons (Connell and Miller 1984). The specific chemical structure and mixtures of these three classes of petroleum hydrocarbons determine their chemical properties, such as solubility and volatility, and persistence and resistance to rates of photochemical oxidation, microbial degradation, and their biological toxicities in the environment (Table 11.2).

11.3.1 ALKANES

The alkanes are chains of carbon atoms with attached hydrogen atoms. They may be simple, straight chains (n-, “normal”), be branched (*iso*-, *sec*-, *tert*-), or have a simple ring configuration (cyclo-) (Figure 11.1). Low molecular weight alkanes have low boiling points and are highly volatile. They are slightly soluble in water but extremely soluble in fats and oils. The lipophilicity enables rapid penetration through membranes and into tissues. High molecular weight alkanes are not soluble in water

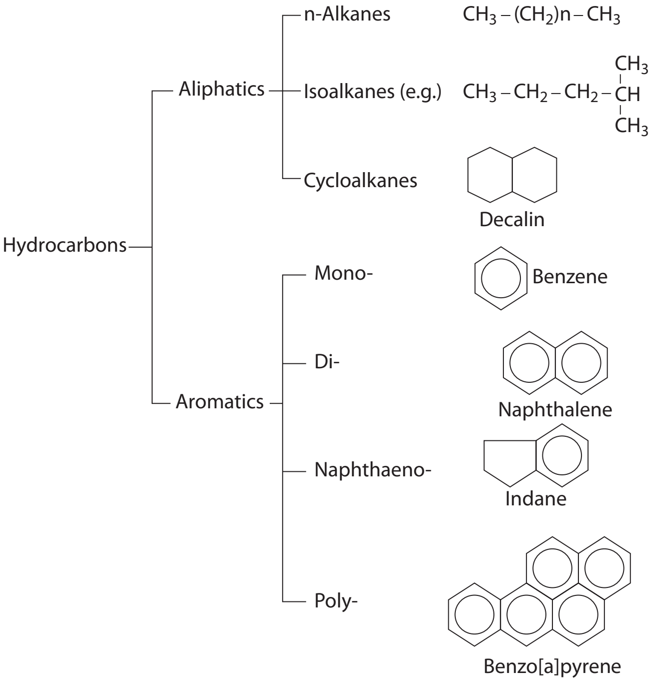

FIGURE 11.1 Some of the common components of crude petroleum.

and are exclusively lipophilic. Low molecular weight alkanes are used as solvents, and degreasers and as thinners and diluents of paints, enamels, varnishes, and lacquers. They are also used as extractants of organic compounds from plant and animal tissues, soils, and sediments and in the production of aviation fuels and gasoline.

11.3.1.1 Health Effect

Alkanes act primarily by solubilizing or emulsifying fats, mucous membranes, and cholesterols. At low concentrations, alkanes are simple irritants and can cause inflammation, redness, itching, and swelling of the skin, mucous membranes, nose, trachea, and bronchioles. They also produce anesthesia and narcosis in the central nervous system (CNS). At high concentrations, acute eczema of the skin and pulmonary edema may develop, as well as unconsciousness or death through asphyxiation by paralysis of the portion of the brain responsible for respiration. Alkanes have also been found to penetrate rapidly into the fatty cells of myelin sheath that surround the nerve fibers, where they dissolve the cells and cause degeneration of the axons, interrupting the transference of nerve impulses (Manahan 1989).

Alkanes of higher molecular weight are considered virtually nontoxic, although they may affect chemical communication and interfere with metabolic processes. Many of the same high molecular weight alkanes are produced biogenically and have been found to occur naturally in marine organisms (Boesch 1974).

Alkanes can be excreted, unaltered, by the lungs and can also be metabolized by the oxidation of the terminal methyl group by molecular oxygen via the mixed-function oxidase (MFO) system to produce an alcohol. Repeated oxidation of the terminal carbon produces an aldehyde and finally a carboxylic acid, which is broken down by β-oxidation to give rise to acetyl coenzyme A as the final product (Atlas and Bartha 1987).

In the atmosphere, low molecular weight alkanes react with the hydroxyl radical (OH·) in a process where a hydrogen atom is abstracted from the alkane to form an alkyl radical. This radical adds molecular oxygen and in the presence of high concentrations of nitrous oxide (NO) forms atmospherically reactive NO_2 (Graedel et al. 1986).

Among the related compounds of alkanes, formaldehyde, an industrial chemical, is widely found in both indoor and outdoor airs. The compound is quite toxic and can cause serious health problems. It can cause nasal cancer, may be linked to leukemia, and worsens asthma and respiratory problems. The toxicity of aldehyde was widely known in the Hurricane Katrina landfall in August 2005. It was reported that frantic Federal Emergency Management Agency (FEMA) officials ordered nearly $2.7 billion in trailers and mobile homes for the victims of the storm to be housed. Manufacturers produced trailers with unusual speed. Reportedly, FEMA's requirements made little mention of the safety of those to be housed. Unfortunately, within months, residents began complaining about unusual sickness, such as breathing problems, burning eyes, noses, and throats. Deaths even occurred. The presence of high concentrations of formaldehyde (an industrial chemical) was found to be in the indoor and outdoor air. It was found to be present in many of the FEMA housing units in amounts exceeding federally recommended limits (TheBellinghamHerald.com 2008).

11.3.2 ALKENES

Alkenes are also chains of carbon atoms with attached hydrogen atoms, but the chains contain carbon–carbon double bonds. They are considered unsaturated in relation to the total possible number of attached hydrogen atoms, compared with an alkane of similar carbon chain length. The double bonds convey a planar configuration to the alkene chains that allows the formation of geometrical isomers *(cis-* and *trans-).* Alkenes are generally more reactive than alkanes but less reactive than aromatics. They are not found in crude petroleum but are present in some refined products, specifically gasoline and aviation fuels. Alkenes undergo addition reactions, forming potentially more toxic metabolites. They can undergo polymerization to create long polyethylene chains, oxidation reactions to form oxides that on hydrolysis can form glycols, and halogenation to form extremely toxic chlorinated and brominated hydrocarbon pesticides.

11.3.2.1 Health Effects

In experimental animals, the *cis-* isomers have been found to cause weakness, nausea, and vomiting due to their adverse effects on the gastrointestinal tract and tremor and cramps due to their effects on the CNS (Manahan 1989).

11.3.3 AROMATIC HYDROCARBONS

Aromatic hydrocarbons have a basic six-carbon atom ring configuration with six hydrogen atoms and three double bonds and are unsaturated in terms of attached hydrogen atoms. The aromatic ring may occur in a single, unattached configuration (as in benzene), as two attached rings (to form naphthalene), or as many attached rings. The multiring structures are termed polycyclic aromatic hydrocarbons (PAH) (Figure 11.1).

The aromatic ring structures may also include substituted methyl and more complex alkyl side chains, as in the case of toluene, the xylenes, cumene, and 2-methylnaphthalene. The substitution of the hydrogen atoms with other compounds yields several distinct chemical species with varying degrees of polarity, lipophilicity, persistence, and toxicity (Rochkind et al. 1986; Manahan 1991). It is, however, the fundamental ring structure unit, with the carbon–carbon resonance stabilized bonds of equal length and energy, that confers great stability to these compounds, making them not only persistent, but also some of the most acutely toxic and carcinogenic compounds in the environment.

Benzene, toluene, and the three isomers of xylene are among the most common monocyclic aromatic compounds found in petroleum (Moore and Ramamoorthy 1984). Compared with other alkanes, alkenes, and PAHs, they have low molecular weight, low water solubility, and high volatility and flammability and have the same toxicological mode of action: narcosis (Manahan 1991; Rappoport 1967), state of stupor, insensibility, or unconsciousness. Their structures, stability, and ability to be both slightly hydrophilic and lipophilic, enhance their accessibility to many niches, species, biochemical pathways, and sites of action. This accounts, in part, for the designation of these compounds as priority pollutants by the EPA (Manahan 1991; Moore and Ramamoorthy 1984).

11.3.3.1 Benzene

Benzene (boiling point 80.1°C) is chemically the most significant aromatic hydrocarbon because it is the starting material for the manufacture of numerous industrial and agricultural products (Manahan 1989, 1991; Moore and Ramamoorthy 1984). It has been in commercial use for over a century, and its toxic effects have been suspected for almost as long. Benzene has a wide variety of uses in industrial society. Some examples include the following:

- an intermediate in the synthesis of pharmaceuticals and other chemicals, such as styrene, detergents, pesticides, and cyclohexane
- a degreasing and cleaning agent
- an antiknock fuel additive (Foo 1991)
- a solvent for extracting pesticides from tissues, soils, and sediments in research and industrial applications
- a thinner and diluent of paints, inks, and lacquers
- a solvent in the rubber industry (Rappoport 1967)

In the atmosphere, benzene and more than 70 of its derivatives are present as a result of combustion of fossil fuels and emissions from a variety of industrial processes. The principal chain reactor is the OH· radical, which can either add to the aromatic ring or abstract a hydrogen atom from the side group. A variety of aromatic aldehydes, alcohols, and nitrates are produced in this way, as well as products of ring

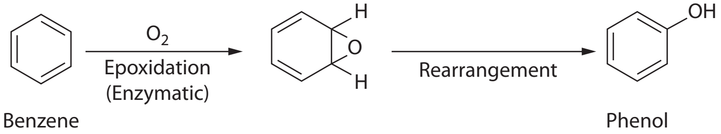

FIGURE 11.2 Biotransformation of benzene.

cleavage. These products have moderately high molecular weight and are moderately soluble in water; therefore, they can be readily deposited on aerosol particle surfaces (Graedel et al. 1986).

The toxicological mode of action of benzene is narcosis, affecting the CNS. At high concentrations, inhalation of air containing approximately 64 g/m^3 of benzene can be fatal within a few minutes, and 1/10 of that level can cause acute poisoning within an hour (Manahan 1989). Exposure causes skin irritation, fluid accumulation in the lungs (edema), excitation, and depression and may eventually lead to respiratory failure and death. At lower concentrations, benzene can cause blood abnormalities, lower white cell count, and bone marrow damage (Manahan 1989; Browning 1953). These toxicological effects have been attributed specifically to the *trans*-benzene-l,2-oxide intermediate formed during eukaryotic oxidative degradation of benzene. During oxidation, the oxygen atom is incorporated directly into the ring, forming an epoxide intermediate (Figure 11.2). The epoxide, which is not immediately degraded, resides in the cell structures and actively reacts with cell nucleophiles, damaging blood, lymph, and bone marrow cells, as well as affecting liver and kidney function. The epoxide is eventually converted to phenol by a slower, nonenzymatic rearrangement process, and the phenol is finally eliminated from the body as its sulfate (Rochkind et al. 1986).

Benzene is of most concern because it is known to be associated with the development of leukemic cancer in humans (Aksoy 1985). Although the mechanisms by which benzene-induced toxicity and induction of leukemia are not yet fully understood, they are known to be complicated by various pathways, including those of metabolism, oxidative stress, DNA damage, cell cycle and growth regulations, and programmed cell death (apoptosis). A report by Yoon et al. (2003) enhanced the knowledge of the mechanisms involved in benzene toxicity. By using mouse bone marrow tissue in their studies, the researchers concluded that the observed effects of benzene on mouse bone marrow cells resulted from

- cellular damage due to benzene metabolites and oxidative stress
- dysfunction of the machinery of cell cycle arrest for repairing damaged DNA, resulting in continuous cycling of damaged cells without even undergoing repair
- inhibition of apoptosis by both disruption of p53-dependent proapoptotic signaling and activation of survival genes
- failure of activating DNA repair genes, which may lead to an increase in cell mutation frequencies at the candidate DNA locus responsible for benzene carcinogenesis, resulting in the development of hemopoietic malignancies

11.3.3.2 Toluene

Toluene (boiling point 110.6°C) is produced primarily as a precursor for the synthesis of other chemicals. For example, 70% of the product is used for the synthesis of benzene, 15% for the manufacture of other chemicals, and 10% as a solvent for paints and as a gasoline additive (Moore and Ramamoorthy 1984; Foo 1991). Toluene is used as one of the major substitutes for benzene because of the extreme hazards associated with benzene exposure.

The toxicological mode of action for toluene is narcosis. At low concentrations, it produces skin irritations, and at higher levels it affects blood cells, the liver, kidney, and CNS (through which it causes headaches, nausea, and impaired coordination) (Manahan 1989). Compared with benzene, toluene is less water soluble and more lipophilic, causing greater concentrations of it to be more rapidly transported to the site of action, so increasing its potential for toxic effects (Kauss and Hutchinson 1975). However, while the methyl group of toluene increases concentration and depressant effects at the site of action, the rapid enzymatic degradation of toluene immediately reduces the concentration, limiting the potential toxicological effects and resulting in a lowered observed toxicity (Berry and Bammer 1977; Kauss and Hutchinson 1975; Donahue et al. 1977). The mechanism involved in moderating the toxic effects is the rapid oxidation of the aliphatic methyl side chain rather than the ring structure. Benzyl alcohol and benzoate intermediates are formed, which are conjugated to hippuric acid (about 70% of the dose is affected) and rapidly eliminated, with the remainder exhaled from the lungs unchanged (Manahan 1989; Rochkind et al. 1986).

11.3.3.3 Xylenes

The xylenes (ortho [*o*-] meta [*m*-] and para [*p*-], boiling points 144.4°C, 139.1°C, and 138.3°C, respectively), have also been used as replacements for benzene and toluene in the production of resins, synthetic fabrics, and plastics and as gasoline additives, cleaners, solvents, and lacquers. As in the case of toluene and benzene, the xylenes act as narcotics on the CNS, causing headaches, impaired coordination, edema, and nausea at higher concentrations and skin irritations, anemia, blood cell damage, and a decrease in blood platelets at lower, chronic exposure levels (Manahan 1989). In oxidative degradation, *m*-xylene and *p*-xylene are metabolized to *m*-toluates and *p*-toluates, which are further oxidized by the meta pathway (Evans et al. 1991; Perry 1979; Worsey and Williams 1975). *o*-Xylene oxidation does occur, but by a modified cometabolic pathway with toluene (Evans et al. 1991). Elimination of xylenes is primarily through the excretion of metabolites in the form of methyl hippuric acid (95% of the absorbed dose), 1–2% as xylenol, and by exhalation of 3–5% as the unchanged solvent.

The double methylation of the xylenes makes them virtually insoluble in water. They are very lipophilic, with the potential for rapid transport to the site of action. As with toluene, the toxicity of the xylenes is mediated by the reduction in their water-soluble fraction concentrations and their rapid biodegradation. The presence of the second methyl group on the benzene ring determines the number of enzymatic steps in the xylene degradation process and the specific pathway, rate of degradation, and potential for bioaccumulation by its location at the *ortho*, *meta*, or *para* position (Berry and Bammer 1977; Kauss and Hutchinson 1975; Evans et al. 1991; Perry 1979; Worsey and Williams 1975).

11.4 POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic or polynuclear aromatic hydrocarbons (PAHs) are a group of compounds composed of two or more fused aromatic rings in linear, angular, or cluster arrangements. By definition, they consist solely of carbon and hydrogen (Baek et al. 1991).

The EPA has focused on the 16 PAHs that are included on the list of 126 priority pollutants (Table 11.3). These were selected on the basis of toxicity, potential for human exposure, and frequency of occurrence at hazardous waste sites.

The reason for the concern over PAHs is that many of them have been shown to be carcinogenic to animals, and substantial data exist incriminating them as carcinogenic to humans (U.S. Department of Health and Human Services [USDHHS] 1990). Eight of the PAHs in Table 11.3 are classified as group B2 compounds, that is, probable human carcinogens. The remaining eight are classified as group D compounds, referring to insufficient data being available to assess their carcinogenic potential. No individual PAH has been classified as belonging to group A (known

TABLE 11.3
Polycyclic Aromatic Hydrocarbons Identified as Priority Pollutants by the U.S. Environmental Protection Agency

Name	Abbreviation	Carcinogenic Classification
Acenaphthylene	Ace	D
Acenaphthene	—	D
Anthracene	Ant	D
Benz(a)anthracene	BaA	B2
Benzo(a)pyrene	BaP	B2
Benzo(b)fluoranthene	BbF	B2
Benzo(k)fluoranthene	BbK	B2
Benzo(g,h,i)perylene	Bpe	B2
Chrysene	Chr	B2
Diben(a,h)anthracene	DbA	D
Fluoranthene	Fth	B2
Fluorene	F1	D
Ideno(1,2,3-c,d)pyrene	IP	B2
Napthalene	Na	D
Phenanthrene	Phe	D
Pyrene	Pyr	D

Note: B2, probable human carcinogens; D, insufficient data are available to assess their carcinogenic potential.

human carcinogens). However, several complex mixtures from which PAHs have been identified are known human carcinogens, such as cigarette smoke, coal tar, and coke oven emissions.

11.4.1 Sources

PAHs are ubiquitous and can occur in the air attached to dust particles or in soil and sediments as solids. PAHs have also been detected in food and water (USDHHS 1990). The predominant source of PAHs is the incomplete combustion of organic material. The anthropogenic sources of PAHs can be divided into stationary and mobile categories of emission (Baek et al. 1991). Vehicular engines are the major contributors to the mobile emissions. The stationary fraction encompasses a wide variety of combustion processes, including residential heating, aluminum production, coke manufacture, incineration (Davies et al. 1976), and power generation. The amounts and types of PAH produced by each of these vary widely due to differences in fuel type and combustion conditions (Baek et al. 1991).

Not all PAHs are the result of human activity. Volcanic eruptions and forest and prairie fires are among the major sources of naturally produced PAHs. In addition, there is some evidence that PAHs may also be formed by direct biosynthesis by microbes and plants (Neff 1979).

11.4.2 Physical and Chemical Properties

As pure chemicals, PAHs generally exist as colorless, white, or pale yellow-green solids. However, the physical and chemical characteristics of PAHs vary with their molecular weight (Neff 1979). Resistance to oxidation and reduction tends to decrease with increasing molecular weight. Vapor pressure and aqueous solubility decrease almost logarithmically with increasing molecular weight. As a consequence of these differences, they tend to be environmentally more stable because they are less amenable to microbial degradation (USEPA 1991).

11.4.3 Transport

PAHs released to the atmosphere are subjected to short- and long-range transit and are removed via wet and dry deposition. In surface water, PAHs can volatilize, photodegrade, oxidize, biodegrade, bind to particulates, or accumulate in an organism. In sediments, PAHs can biodegrade or accumulate in aquatic organisms. PAHs in soils can biodegrade or accumulate in plants; PAHs can enter the groundwater and be transported within an aquifer. Figure 11.3 illustrates major routes involved in the transport of PAHs.

11.4.4 Exposure

PAHs are widely distributed in the environment. They have been detected in air, water, soil, sediment, food, and other consumer products such as cosmetics and cigarettes. As a result, humans are exposed to these chemicals as part of everyday living.

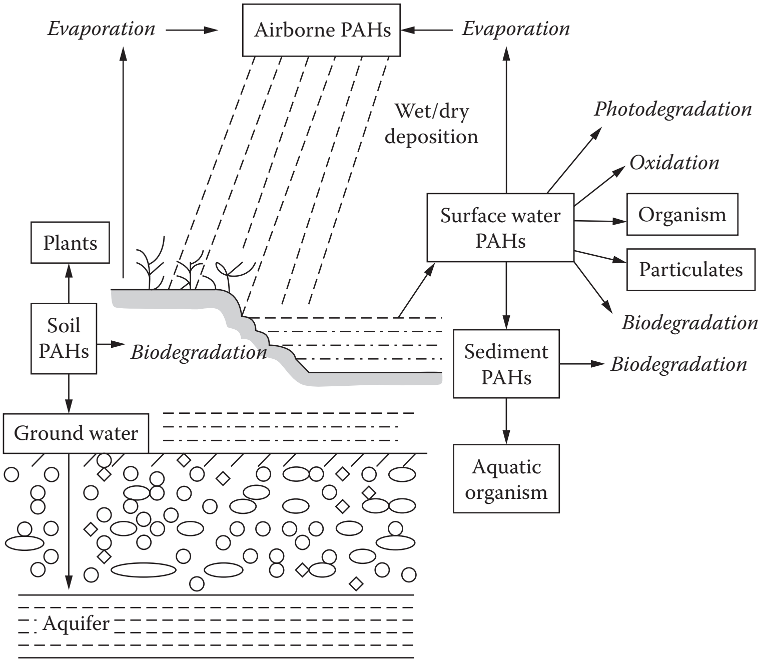
Evaporation
Airborne PAHs
Evaporation
Wet/dry deposition
Photodegradation
Oxidation
Surface water PAHs
Organism
Particulates
Plants
Soil PAHs
Biodegradation
Biodegradation
Sediment PAHs
Biodegradation
Ground water
Aquatic organism
Aquifer

FIGURE 11.3 Transport of PAHs.

As stated previously, most of the direct releases of PAHs into the environment are to the atmosphere. Important sources of PAHs in surface waters include deposition of airborne PAHs, municipal wastewater discharge, urban storm water runoff, and industrial discharges (USEPA 1991). Most of the PAHs in surface waters and soils are believed to arise from atmospheric deposition. Food groups that tend to have the highest levels of PAHs include charcoal-broiled or smoked meats, leafy vegetables, grains, and vegetable fats and oils (Menzie et al. 1992). The presence of PAHs on leafy vegetables and grains is believed to be caused by atmospheric deposition and reflects local conditions in the growing area. The average American is estimated to consume between 1 and 5 μg/day of carcinogenic PAHs, with unprocessed grains and cooked meats as the greatest source of these substances (carcinogenic being defined as group B2 compounds) (Menzie et al. 1992). A person who consumes a heavy meat diet has the highest estimated potential dose, on the order of 6 to 12 μg/day. A vegetarian diet can offer an elevated PAH intake of 3 to 9 μg/day if the diet comprises leafy vegetables, such as lettuce and spinach, and unrefined grains.

Using the EPA assumption of a respiration rate of 20 m^3/day, the estimated potential PAH dose by inhalation by nonsmokers ranges between 0.02 and 3 μg/day, with a median value of 0.16 μg/day (Menzie et al. 1992). Tobacco smoke can be a major source of airborne carcinogenic PAHs. Mainstream smoke from unfiltered cigarettes may contain 0.1 to 0.25 μg/cigarette. An individual who smokes one pack of unfiltered cigarettes a day is estimated to inhale an additional 2 to 5 μg/day (Appendix 3). Indoor air levels associated with tobacco smoke have been reported in the range of 3 to 29 ng/m^3. The consequence is that exposure to secondary cigarette smoke may be implicated in adverse health effects.

The potential dose of carcinogenic PAHs from drinking water (assuming an average drinking water consumption of 2 L/day) ranges between 0. 2 and 120 ng/day, with a median value of 6 ng/day (USEPA 1991). Drinking water concentrations of PAHs have been reported to range between 0.1 and 61.6 ng/L, with most drinking water values falling between 1 and 10 ng/L.

Carcinogenic PAHs are found in all surface soils (Menzie et al. 1992), with urban areas having higher concentrations than do agricultural and forest soils. Typical concentrations of carcinogenic PAHs are in the range of 5 to 100 μg/kg. Agricultural soils contain 10 to 100 μg/kg, and urban soils contain 0.6 to 3 mg/kg. Assuming a rate of incidental ingestion of soil of 50 mg per day, the potential intake of carcinogenic PAHs for urban populations ranges from 0.003 to 0.4 μg/day, with the median value 0.06 μg/day.

Excluding occupational exposure routes, food may be the major source of carcinogenic PAHs for nonsmokers. Smokers of nonfiltered cigarettes may be exposed to twice the concentration of carcinogenic PAHs.

11.4.5 Metabolism and Toxicity

PAHs enter the human body quickly by all routes of exposure: inhalation, ingestion, and dermal contact (USDHHS 1990). The rate of absorption is increased when they are present in mixtures. PAHs are conveyed to all the tissues of the body containing fat and tend to be stored mostly in the liver and kidney, with smaller amounts in the spleen, adrenal glands, and ovaries. Results from animal studies showed that PAHs tend not to be stored in the body for prolonged periods and are usually excreted within a matter of days.

The lipophilicity of PAHs enables them to readily penetrate cellular membranes. Subsequent metabolism renders them more water soluble and thus more readily removed from the body. However, PAHs can also be converted to more toxic or carcinogenic metabolites. One factor that may influence the delicate balance of toxification and detoxification is the site at which the chemically reactive metabolite is formed. Metabolism of PAHs occurs in all tissues (Neff 1979). The extent of induction of enzyme systems following exposure to xenobiotics is known to vary with tissues. For example, more induction generally occurs in the liver than in the lung or skin.

In mammals, the cytochrome P450 MFO system is responsible for initiating the metabolism of xenobiotics. As discussed in Chapter 6, the primary function of this system is to render lipophilic compounds more water soluble. Although this system effectively metabolizes certain xenobiotics, others, such as PAHs, are transformed into intermediates that are highly toxic, mutagenic, or carcinogenic to the host. For example, oxidative metabolism of benzo(a)pyrene by the MFO system converts it into a dihydroxy epoxide, believed to be a carcinogen that can interact with DNA (see Chapter 17).

The PAHs are resistant to degradation due to their complex ring structures. As a result, these compounds have the potential to recycle and participate in atmospheric

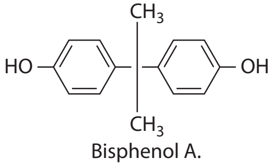

FIGURE 11.4 Bisphenol A.

reactions several times before being degraded enough to be removed from the environment. The same resistance and persistence of these chemicals occur in terrestrial and aquatic systems, making these compounds the most hazardous in terms of long-term, chronic exposure to their carcinogenic and mutagenic properties. Furthermore, recent studies showed that the toxicity of PAHs increases following photomodification by natural sunlight (Huang et al. 1995).

Controversies about the safety of bisphenol A (BPA) (Figure 11.4) have been voiced for several years. Those promoting the banning of the compound stress the toxic effect of the compound being used to coat food cans. The Food and Drug Administration (FDA) has had a difficult balancing act in regulating human exposure to the chemical.

A group of scientists in the American Chemical Society claim that "for many food applications, for example, in the metal-packaging industry, finding a new material with just the right combination of properties remains a major challenge" because the materials used to coat food cans must adhere strongly, provide corrosion resistance, and withstand the high temperatures required for sterilization and processing (Baum 2010). According to Voith (2009):

> Linings made with BPA give a wide range of canned goods their long shelf life and good food safety record. Without any lining, a typical aluminum or steel can creates a strong air and light barrier all by itself. But eventually, contact between the food and metal will corrode the packaging, leading to spoilage or microbial contamination. Corrosion would rapidly ruin high-acid foods such as tomatoes. Low-acid foods like peas may last longer but are more likely to harbor toxin-producing bacteria such as *Clostridium botulinum*.

Alternatives to BPA-based linings do not perform as well or are significantly more expensive.

Furthermore, the evidence linking BPA with adverse health effects is considered to be weak. Many studies have been carried out, and the results have been contradictory. This is why the FDA has acted cautiously with regard to BPA and why the chemical and food-packaging industries resist stringent regulation of it. The FDA announced in 2010 that it has "some concern" about the potential health effects of BPA in infants and children, but also said that more research was needed to fully assess the safety of the chemical. Market pressure, however, has effectively removed BPA from products such as baby bottles, so that is no longer an issue.

According to Baum (2010), *C&EN* editor in chief, "No one has shown that adults exposed to BPA at the levels that leach from food container liners suffer any harm. Potential replacements for BPA don't work as well and very likely will pose risks of their own."

REVIEW QUESTIONS

1. What are VOCs? What are the major groups contained in VOCs?
2. What are some of the major anthropogenic sources of VOCs?
3. Chemically, what are the three major classes of petroleum?
4. What is narcosis? What types of VOCs can cause it?
5. What is the health effect of low concentrations of benzene?
6. How is benzene metabolized in the body? In what form is it excreted?
7. Name three environmentally important monoaromatic compounds.
8. What is the concern over PAHs?
9. What kind of foods contain relatively high levels of PAHs?
10. Briefly explain the fate of the PAHs in surface water.
11. Explain the process involved in the metabolism of benzo[a]pyrene and indicate the consequence of its metabolism.

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12 Soil and Water Pollution

Environmental Metals and Metalloids

12.1 INTRODUCTION

The metals found in our environment are derived from a variety of sources, such as the natural weathering processes of Earth's crust, mining, soil erosion, industrial discharge, urban runoff, sewage effluents, pest or disease control agents applied to plants, air pollution fallout, and a number of other sources (Fargasova 1994). Since the Industrial Revolution, the use of metals has been a mainstay of the economy in many developed countries, particularly the United States. However, with the increase of mining for metal ores, as well as the combustion of coal as an important energy source in many countries, health and exposure risks to workers and the general public have become of increasing concern.

While some metals found in our environment are essential nutritionally, others are not. The latter include some "heavy metals," a group of metallic elements that exhibit certain chemical and electrical properties and are generally those having a density greater than 5 g/cm^3 (Forstner and Wittmann 1979). These metals exceed the atomic mass of calcium (Ca). Most of the heavy metals are extremely toxic because, as ions or in certain compounds, they are soluble in water and can be readily absorbed into plant or animal tissue. After absorption, these metals tend to bind to biomolecules such as proteins and nucleic acids, impairing their functions.

The effects of toxic heavy metals on living organisms have for a long time been considered almost exclusively a problem of exposed industrial workers and of accidental childhood poisonings. Until recently, much of the literature concerning the subject, therefore, deals with experiments on children's exposure to lead paint. Although significant progress has been made in reducing the levels of a number of toxic metals in our environment, as exemplified by the marked reduction in atmospheric lead (Pb) pollution since the 1980s, problems with heavy metals still exist in many parts of the world. According to the U.S. Centers for Disease Control and Prevention (CDC), Pb poisoning is the most common and serious environmental disease affecting young children.

In this chapter, the sources and the health and biological effects of several metals and a metalloid on living organisms are discussed. The discussion will include Pb, cadmium (Cd), mercury (Hg), nickel (Ni), and arsenic (As). These and a number of other metals are widely used in industry, and Pb, Cd, and Hg, in particular, are generally considered the most toxic metals to humans and animals.

12.2 LEAD

12.2.1 Characteristics and Use

Lead occurs naturally, in small amounts, in the air, surface waters, soil, and rocks. Because of its unique properties, Pb has been used for thousands of years. Its high ductility (the quality of being ductile, i.e., capable of being permanently drawn out without breaking) and malleability have made Pb the choice for a large number of materials, including glass, paint, pipes, building materials, art sculptures, print typefaces, weapons, and even money. The use of Pb has accelerated since the Industrial Revolution, particularly since World War II. However, its wide use has resulted in elevated Pb concentrations in the ecosystem. For example, in locations where Pb is mined, smelted, and refined; where industries use the metal; and in urban–suburban complexes, the environmental Pb levels are greatly increased. Until recently, in many countries the primary source of environmental Pb has been the combustion of leaded gasoline.

Lead has a low melting point of 327°C. It is extremely stable in compound forms. Therefore, dangerous forms may remain in the environment for a long time. This stability made it the number one choice for high-quality paint because it resisted cracking and peeling and retained color well. Millions of tons of lead-based paint were used in the United States before it was banned in 1978. (Note: Europe banned the use of Pb in residences in 1921.) Because Pb is ubiquitous and is toxic to humans at high doses, levels of exposure encountered by some population groups constitute a serious public health problem (National Research Council [NRC] 1980). The importance of Pb as an environmental pollutant is obvious since the U.S. Environmental Protection Agency (USEPA) has designated the metal as one of the six criteria air pollutants.

12.2.2 Sources of Lead Exposure

12.2.2.1 Airborne Lead

Airborne Pb pollution is a growing problem facing many countries. Early Pb poisoning outbreaks were associated with the burning of battery shell casings. Industrial emissions of Pb also became a concern as the Industrial Revolution progressed. Increasing Pb pollution in the environment was first revealed in a 1954 study conducted by a group of scientists from the U.S. and Japan on the Pb contents of an arctic snow pack in Greenland. In the study, the scientists found steady increases in Pb levels, beginning about the year 1750. Sharp increases were evident after the end of World War II. Importantly, the content of other minerals in the snowpack was found to remain steady. These observations suggest that increasing atmospheric Pb pollution is a consequence of human activities (Murozumi et al. 1969).

The main industrial sources of Pb pollution include smelters, refineries, incinerators, power plants, and manufacturing and recycling operations. For example, Kellogg, a small town in Idaho, lies in a deep valley directly downwind of the Bunker Hill lead smelter. Beginning in 1974, about 200 children between the ages of 1 and 9 years were screened annually for blood Pb levels. Until the closure of the plant in 1983, after 100 years of operation, Kellogg children's blood Pb levels were among the highest in the United States. Since the plant closed, screenings showed a steady decrease in children's blood Pb levels. In 1986, the average level was about the same as in children who had not lived near a smelter, with most levels falling below the established action level of 25 μg/dL (Anon 1991).

Until recently, the number one factor contributing to Pb air pollution was, however, the automobile. The addition of tetraethyl lead as an antiknock agent in gasoline led to a steep increase in Pb emission. During combustion, Pb alkyls decompose into lead oxides, which react with halogen scavengers (used as additives in gasoline), forming lead halides. Ultimately, these compounds decompose to lead carbonate and oxides. However, a certain amount of organic Pb is emitted from the exhaust. It was estimated earlier that about 90% of the atmospheric Pb was due to automobile exhaust, and that worldwide a total of about 400 tons of particulate Pb was emitted daily into the atmosphere from gasoline combustion. Since the mandatory use of unleaded gasoline in the United States began in 1978, followed by improved industrial emission control, atmospheric Pb emission from major sources in the United States has decreased dramatically. According to the EPA, annual Pb emission from major emission sources in the United States decreased from 56,000 tons in 1981 to 7,100 tons in 1990 (USEPA 1991).

Encouragingly, since the beginning of the twenty-first century, Pb exposure in adults has decreased substantially in the United States (Gelberg and DePersis 2009). Nevertheless, almost 10,000 adults with blood Pb levels of 25 μg/dL or greater were reported from 37 states that provided data to CDC's Adult Blood Lead Epidemiology and Surveillance (ABLES) program in 2003 and 2004. While atmospheric Pb pollution has also decreased in other developed countries, a similar trend has not been shown in many less-developed countries. This is particularly true in some of those countries that have achieved marked economic development.

12.2.2.2 Waterborne Lead

Although Pb emissions into the environment have declined markedly as a result of the decreased use of leaded gasoline, Pb is still a potential problem in aquatic systems because of its industrial importance. Once emitted into the atmosphere or soil, Pb can find its way into the aquatic systems. Both surface water and groundwater may contain significant amounts of Pb derived from these sources.

Water is the second-largest source of Pb for children (Pb in paint being the largest). In 1992, the levels of Pb in 130 of the nation's 660 largest municipal water systems, serving about 32 million people, were found to exceed the "action level" of 15 ppb set by the EPA. Many homes are served by Pb service lines or have interior pipes of Pb or copper (Cu) with Pb solder (American Chemical Society [ACS] 1992).

Another serious problem is related to waterborne Pb from lead shot left in lakes and ponds. Although nonlead shot is now in use, much lead shot remains in aquatic systems. A large number of waterfowl in the United States are poisoned or killed annually as a result of ingesting lead shots. For example, according to a bird rehabilitation center in Whatcom County, Washington, lead shot killed nearly 1,000 swans in the county and adjacent areas in British Columbia, Canada, in the 5 years following the center's opening. The investigators at the center indicated they were unable to pinpoint the source of the lead shot that had killed the birds.

12.2.2.3 Lead in Food

Food is a major source of Pb intake for humans and animals. Plant food may be contaminated with Pb through its uptake from ambient air and soil. Animals may ingest Pb-contaminated vegetation. In humans, Pb ingestion may arise from eating Pb-contaminated vegetation or animal foods. Vegetation growing near highways has long been known to accumulate high quantities of Pb from automobile exhaust (Khalid et al. 1996). However, recent studies show that in the United States the levels of Pb in such vegetation have decreased significantly following the general use of unleaded gasoline. Another source of ingestion is through the use of Pb-containing vessels or Pb-based pottery glazes.

Lead paint had been used until 1978, when it was banned in common use. Of 129 million U.S. housing units, 76.5 million were built before 1980, according to the Census Bureau (Lin-Fu 1982). The eventual deterioration of these houses continues to cause exposure of children to Pb. Young children eat flaking paint from the walls of these houses—a phenomenon called *pica*. The risk of this practice to children has been widely recognized.

12.2.2.4 Lead in Soils

Almost all of the Pb in soil comes from Pb-based paint chips flaking from homes, factory pollution, and the use of leaded gasoline. In the United States, emission of Pb through various uses of the metal is estimated at 600,000 tons/year. Countless additional tons are dispersed through mining, smelting, manufacturing, and recycling. Disposal of Pb paint is a further cause of soil contamination, as is the use of Pb in insecticides. Earlier studies showed that about 50% of the Pb emitted from motor vehicles in the United States was deposited within 30 m of the roadways, with the remainder scattered over large areas (Ryan 1976). Lead tends to stick to organic matter in soils; most of the Pb is retained in the top several centimeters of soils, where it can remain for years. Soil contamination also leads to other problems associated with Pb-contaminated foods.

12.2.3 LEAD TOXICITY

12.2.3.1 Lead Toxicity to Plants

Plants can absorb and accumulate Pb directly from ambient air and soils. Lead toxicity to plants varies with plant species and the other trace metals present. For example, barley plants are very sensitive to Pb (Oberlander and Roth 1978). Lead has been shown to inhibit seed germination by suppressing general growth and root elongation (Koeppe 1977; Yu 1991). The inhibitory effect of Pb on germination, however, is not as severe as that exhibited by several other metals. For example, in a study on the effect of Cr, Cd, Hg, Pb, and As on the germination of mustard seeds *(Sinapis alba),* Fargasova (1994) showed that after 72 hours the most toxic metal for seed germination was As^{5+}, while the least toxic was Pb^{2+}. According to Koeppe (1977), Pb might be bound to the outer surface of plant roots, as crystalline or amorphous deposits, and could also be sequestrated in the cell walls or deposited in vesicles. This might explain the higher concentrations of Pb in roots (Lyngby and Brix 1984) and can explain the low toxic effect on mustard seeds.

Pb may be transported in plants following uptake and can decrease cell division, even at very low concentrations. Koeppe and Miller (1970) showed that Pb inhibited electron transport in corn mitochondria, especially when phosphate was present.

12.2.3.2 Lead Poisoning in Animals and Fish

Young animals have been shown to be more susceptible to Pb poisoning than older animals. For example, growing rats accumulated more Pb in their bones than did adult rats, and 1-week-old suckling rats absorbed Pb from their intestinal tract much more readily than adults (Kostial et al. 1971; Forbes and Reina 1972).

In aquatic systems, acidification of waters is an important factor determining Pb toxicity. Eggs and larvae of common carp (*Cyprinus carpid*) exposed to Pb at pH 7.5 showed no significant differences in mortality compared to the control. At pH 5.6, again there was no significant mortality in the Pb-exposed eggs, but the larvae did show significant mortality at all treatment levels. In addition, a marked change in the swimming behavior was observed with the exposed larvae, and a majority of them were seen lying at the bottom of the test chamber, in contrast to the free-swimming controls. Lead exposure also influenced heartbeat and tail movements, with increasing heart rate and decreasing tail movements with increase in Pb concentrations. Subsequent studies showed that Pb uptake and accumulation increased with decreasing pH values (Stouthart et al. 1994). The influence of Pb on freshwater fish also varies with exposed species. For instance, goldfish are relatively resistant to Pb, which may be due to their profuse gill secretion.

As mentioned previously, ingestion of expended Pb shots in lakes and in the field has resulted in death of a large number of birds each year in the United States. Pb absorbed by the bird paralyzes the gizzard, and death follows as a result of starvation.

Three condors in California were found dead in northern Arizona in January 2010, according to local newspapers. They died as a result of ingesting Pb pellets while they were feeding on carrion. Condor recovery program officials said that the deaths were the first from Pb poisoning in 3 years among condors in Arizona and Utah. Condors feed on dead animals, often big game killed by hunters or the entrails left behind when the game are field dressed. About 350 condors are alive today, with about half in captive breeding programs.

12.2.3.3 Health Effects of Lead in Humans

In humans, about 20% to 50% of inhaled and 5% to 15% of ingested inorganic Pb is absorbed. In contrast, about 80% of inhaled organic Pb is absorbed, whereas ingested organic Pb is absorbed readily. Pb ingestion in the United States is estimated to range from 20 to 400 μg/day. An adult absorbs about 10% of ingested Pb, whereas in children the value may be as high as 50%. Once in the bloodstream, Pb is primarily distributed among blood, soft tissue, and mineralizing tissue (Figure 12.1). The bones and teeth of adults contain more than 95% of the total body burden of Pb. In times of stress, the body can metabolize Pb stores, thereby increasing its levels in the bloodstream. Lead is accumulated over a lifetime and released very slowly. In single-exposure studies with adults, Pb has a half-life ($T_{1/2}$) in blood of approximately 25 days. In soft tissue, the half-life is about 40 days, and in the nonlabile portion of bone, it is more than 25 years.

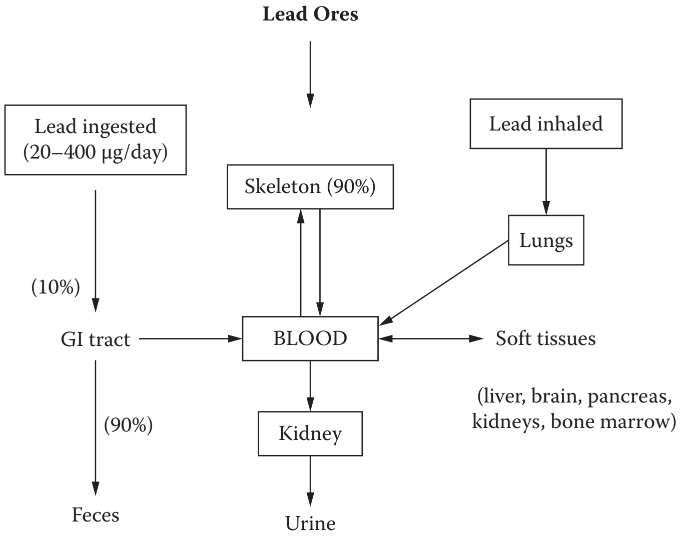

FIGURE 12.1 Lead metabolism in humans. GI, gastrointestinal.

Lead toxicity has been known for over 2,000 years. The early Greeks used Pb as a glaze for ceramic pottery and became aware of its harmful effects when it was used in the presence of acidic foods. Researchers suggested that some Roman emperors became ill, and even died, as a result of Pb poisoning from drinking wines contaminated with high levels of Pb.

According to a study (Ryan et al. 2004), Ludwig van Beethoven died from Pb poisoning after 4 months of misery on a dirty straw mattress in Vienna, Austria. The composer's poisoning by Pb was revealed by the high levels of Pb in hair, as noted by Christian Reiter, forensic scientist of the Medical University of Vienna. It was theorized that Beethoven's doctor Warruch, while treating Beethoven for pneumonia, administered a medicine containing Pb, as many medicines did at the time. Because hair grows at a measurable rate, it traces a time line. And, because Pb and other toxins migrate from the bloodstream to the hair and remain there, forensic researchers study hair for clues about sickness and sociopathic behavior. Beethoven suffered from depression, deafness, digestive troubles, and other ailments, making him an ideal subject (Ryan et al. 2004).

Lead is found in all human tissues and organs, although it is not needed nutritionally. It is known as one of the *systemic poisons* because, once absorbed into the circulation, it is distributed throughout the body. Pb can then affect various organs and tissues. It inhibits hematopoiesis (formation of blood or blood cells within the living body) because it interferes with heme synthesis (see Section 12.2.4), and Pb poisoning may cause anemia. Pb also affects the kidneys by causing renal tubular dysfunction. This, in turn, may lead to secondary effects. The effects of Pb on the gastrointestinal tract include nausea, anorexia, and severe abdominal cramps (lead colic) associated with constipation. Pb poisoning is also manifested by muscle aches and joint pain, lung damage, difficulty in breathing, and diseases such as asthma, bronchitis, and pneumonia. Pb poisoning can also damage the immune system, interfering with cell

TABLE 12.1
Risks of Lead

For Children	For Adults
Lowered IQ	Memory and concentration problems
Learning and behavioral difficulties	Nerve disorders
Slowed growth	Reproductive problems
Headache	Muscle and joint pain
Hearing problems	Hypertension (high blood pressure)

Source: U.S. Environmental Protection Agency.

maturation and skeletal growth. Pb can pass the placental barrier and may reach the fetus, causing miscarriage, abortions, and stillbirths.

According to the CDC, lead poisoning is the most common and serious environmental disease affecting young children (U.S. Department of Health and Human Services [USDHHS] 2002). Children are much more vulnerable to Pb exposure than adults because of their more rapid growth rate and metabolism. Pd absorption from the gastrointestinal tract in children is also higher than in adults (25% vs. 8%), and ingested Pb is distributed to smaller tissue mass. Children also tend to play and breathe closer to the ground, where Pb dust concentrates. One particular problem has been the Pb poisoning of children who ingest flakes of Pb-based paint. This type of exposure accounts for as much as 90% of childhood Pb poisoning. The main health concern in children is retardation and brain damage. High exposure may be fatal.

The developing fetus is also highly susceptible to Pb. According to the Public Health Service, in 1984 more than 400,000 fetuses were exposed to Pb through maternal blood (U.S. Department of Health and Human Services 1992). Pb is associated with early developmental defects, and the developing nervous system in children can be adversely affected at blood Pb levels less than 10 μg/dL.

The primary target organ for Pb is the central nervous system (CNS). Pb can cause permanent damage to the brain and nervous system, resulting in such problems as retardation and behavioral changes (see Table 12.1).

Of greatest current concern is the impairment of cognitive and behavioral development in infants and young children. Because of this, the CDC lowered the definition of elevated blood Pb level for children under age 6, bringing it from 25 μg Pb/dL to 10 μg Pb/dL (Ryan et al. 2004). The median levels in children under age 6 decreased from about 15 to 18 μg Pb/dL blood in 1970 to 2 to 3 μg Pb/dL in 1994 as a result of the concurrent reduction of Pb in automotive emissions, paint, drinking water, and soldered food cans. However, more than 2.2% of children age 1 to 5 years still have blood Pb concentrations above 10 μg/dL. Statistics also show that 17% of children in the United States are at risk of Pb poisoning.

Studies showed that Pb has harmful effects on juvenile behavior. Researchers collected data from as early as 1979 when pregnant women and their healthy babies regularly had their blood drawn at four medical clinics. They then tracked down 250 of the subjects, ages 19 to 24. Factoring in parental IQ, education, income, and drug use, they found that the more Pb in a child's blood from birth through age 7, the more likely he or she was arrested as an adult. The tie between high Pb and violent crime was found to be particularly strong (Barton et al. 1978). Similar evidence was presented by researchers at the University of Cincinnati, who released new evidence that draws a direct relationship between the amount of Pb in a child's blood and the likelihood he or she will commit crimes as an adult (*USA Today* March 2010).

New EPA rules require more Pb paint safety. More than a million American children a year are at risk of being poisoned by lead-based paint in their homes, leading to learning disorders and behavioral problems. according to the EPA. A federal rule that took effect on February 22, 2010, forces contractors to use "lead-safe" practices when working on homes, day-care centers, and schools built before 1978, the year lead paint was banned for residential use because of health risks.

Although Pb is no longer allowed in car or truck fuel or in many paint products, it can be picked up from soil near homes or roads or from drinking water in old lead pipes. A tragedy was reported by the CDC as "unprecedented" in its work with Pb poisoning worldwide. The incident occurred in impoverished Nigerian villages contaminated with Pb, particularly in the village of Zamfara, where 160 children died and hundreds became ill. Some of the children could not stand, and some went blind or deaf. Doctors originally suspected that the tragedy was caused by malaria. They were found wrong after blood tests revealed that the real killer was Pb unearthed by villagers digging for gold. The gold ore, containing high levels of Pb, was brought back to the villages to be stored and broken to extract the gold. The rocks were often chiseled into smaller pieces as the young children played nearby. Dust and flakes accumulated in the villages' communal areas, which children ran through. Following the discovery, cleanup work was started.

According to the International Agency for Research on Cancer (IARC), lead acetate ($[CH_3COO]_2Pb$), and lead phosphate ($Pb_3[PO_4]_2$) are designated as "reasonably anticipated to be human carcinogen[s]" based on sufficient evidence of carcinogenicity in animal experiments. When administered in the diet of rats, lead acetate induced renal adenomas and carcinomas and cerebral gliomas. Subcutaneous injections of lead phosphate induced renal cortical tumors. However, there is inadequate evidence for the carcinogenicity of lead acetate and lead phosphate in humans (Hernberg et al. 1970).

12.2.4 Biological Effects of Lead

In plants, Pb has been shown to inhibit the electron transport in corn mitochondria (Koeppe and Miller 1970), to depress the respiratory rate in germinating seeds, and to inhibit various enzyme systems.

As a systemic poison, Pb can cause many adverse effects in different tissues. It may be expected that these abnormalities are related to biochemical changes. Although the mechanisms involved in Pb toxicity are complex, several examples are given.

As an electropositive metal, Pb has a high affinity for the sulfhydryl (SH) group. As discussed in Chapter 4, an enzyme that depends on the SH group as the active site will be inhibited by Pb. In this case, Pb reacts with the SH group on the enzyme molecule to form mercaptide, leading to enzyme inhibition. Reaction 12.1 shows the chemical reaction between the Pb^{2+} ion and two SH-containing molecules:

$$2RSH + Pb^{2+} \rightarrow R{-}S{-}Pb{-}S{-}R + 2H^{+} \qquad (12.1)$$

Examples of the SH-dependent enzymes include adenyl cyclase and aminotransferase. Adenyl cyclase catalyzes the conversion of adenosine triphosphate (ATP) to the cyclic adenosine monophosphate (cAMP) needed in brain neurotransmission. Aminotransferase is involved in transamination and thus is important in amino acid and protein metabolism.

Because the divalent Pb^{2+} ion is similar in many ways to the Ca^{2+} ion, Pb may exert competitive action in processes such as mitochondrial respiration and neurological functions. In mammals, Pb can compete with Ca for entry at the presynaptic receptor. Because Ca evokes the release of acetylcholine (ACh) across the synapse (see Chapter 13), this inhibition manifests itself in the form of decreased end-plate potential. The miniature end-plate potential release of subthreshold levels of ACh is shown to be increased (Barton et al. 1978). The chemical similarity between Pb and Ca may partially account for the fact that they seem interchangeable in biological systems, and that 90% or more of the total body burden of Pb is found in the skeleton.

Lead causes adverse effects on nucleic acids, leading to either decreased or increased protein synthesis. Pb has been shown to decrease amino acid acceptance by transfer RNA (tRNA), as well as the ability of tRNA to bind to ribosomes. Pb also causes disassociation of ribosomes. The effects of Pb on nucleic acids therefore have important biological implications (Hernberg et al. 1970).

One of the most widely known biochemical effects of Pb is the inhibition of δ-aminolevulinic acid dehydratase (ALA-D) (Tephly et al. 1978). and ferrochelatase (Cherlewski 1979), two key enzymes involved in heme biosynthesis. ALA-D is responsible for the conversion of δ-atninolevulinic acid into porphobilinogen (PBG), whereas ferrochelatase catalyzes the incorporation of Fe^{2+} into protoporphyrin IX to form heme (Figure 12.2). Inhibition of these two enzymes by Pb therefore severely impairs heme synthesis. ALA-D inhibition by Pb is readily exhibited since the enzyme activity is closely correlated with blood Pb levels. An increased excretion of δ-aminolevulinic acid in urine provides evidence of increased Pb exposure. A concomitant decrease in blood porphobilinogen concentrations also occurs. These observations have been utilized in experimental and clinical laboratory studies involving Pb poisoning.

12.2.5 LEAD TOXICITY AND NUTRITION

Nutritional factors can influence the toxicity of Pb in humans by altering its absorption, metabolism, or excretion. Several nutrients affect the absorption of Pb from the gastrointestinal tract. These include Ca, phosphorus (P), iron (Fe), lactose, fat, and vitamins C, D, and E. Low intakes of Ca, P, and Fe, for example, may increase Pb absorption (USDHHS 2002) or decrease Pb excretion, resulting in higher toxicity, while a high-fat intake may lead to increased Pb accumulation in several body tissues.

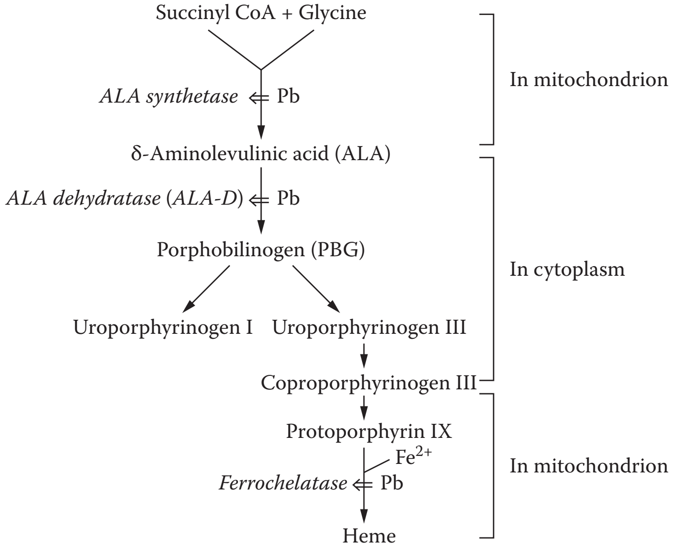
Succinyl CoA + Glycine
ALA synthetase ⇐ Pb
δ-Aminolevulinic acid (ALA)
ALA dehydratase (ALA-D) ⇐ Pb
Porphobilinogen (PBG)
Uroporphyrinogen I
Uroporphyrinogen III
Coproporphyrinogen III
Protoporphyrin IX
Fe2+
Ferrochelatase ⇐ Pb
Heme
In mitochondrion
In cytoplasm
In mitochondrion

FIGURE 12.2 Lead inhibition of heme synthesis. ⇐, site of Pb inhibition.

Calcium, P, and Fe are shown to decrease Pb absorption. Competition for mucosal binding proteins is one mechanism by which Ca reduces the intestinal absorption of Pb. The absorption of Pb is increased in Fe-deficient animals. Thus, Fe deficiency may contribute to the incidence of Pb poisoning in exposed persons. Other nutrients, such as zinc (Zn) and magnesium (Mg), also affect the metabolism of Pb, especially the placental transfer of Pb from pregnant mother to fetus (Cherlewski 1979).

The effect of vitamin C on Pb toxicity appears to be complex. Whereas both vitamin C and vitamin D increase Pb absorption, vitamin C may also lower Pb toxicity. Vitamin E also affects Pb toxicity. In the blood, Pb can react directly with the red blood cell membrane, causing it to become fragile and more susceptible to hemolysis. This may result in anemia. Splenomegaly (enlargement of the spleen) occurs when the less-flexible red blood cells become trapped in the spleen. It is suggested that Pb may mark the red blood cells as abnormal and contribute to splenic destruction of the cells. Lead may act as an oxidant, causing increased lipid peroxidation damage. As noted, vitamin E is an antioxidant and can limit the peroxidation process and damage. Less-severe anemia and splenomegaly are observed in Pb-poisoned rats fed diets containing supplemental vitamin E.

12.3 CADMIUM

12.3.1 Introduction

The outbreak of *itai-itai-byo* or "ouch-ouch disease" in Japan was the historical event that for the first time drew the world's attention to the environmental hazards of Cd poisoning. In 1945, Japanese farmers living downstream from the Kamioka Zinc-Cadmium-Lead mine began to suffer from pains in the back and legs, with fractures, decalcification, and skeletal deformation in advanced cases (Buckler et al. 1986). The disease was correlated with high Cd levels in rice produced from rice paddies that were irrigated by contaminated stream water. The residents' drinking water was also highly polluted (Gloag 1981).

The increased use of Cd and emissions from its production, as well as Pb and steel production, burning of fossil fuel, use of phosphate fertilizers, and waste disposal in the past several decades, combined with the long-term persistence of Cd in the environment, have reinforced the concern aroused by itai-itai-byo. Indeed, many researchers consider Cd to be one of the most toxic trace elements in the environment. Plants, animals, and humans are exposed to the toxicity of this metal, in different but similar ways. Like other heavy metals, Cd binds rapidly to extracellular and intracellular proteins, thus disrupting membrane and cell function (USEPA 1980).

12.3.2 Characteristics and Use of Cadmium

Cadmium is a nonessential trace element and is present in air, water, and food. It is a silver-white metal with an atomic weight of 112.4 and a low melting point of 321°C. As a metal, Cd is rare and not found in a pure state in nature. It is a constituent of smithsonite ($ZnCO_3$) and is obtained as a by-product from the smelting of Zn, Pb, and Cu ores.

A distinctive characteristic of Cd is that it is malleable and can be rolled into sheets. The metal combines with the majority of other heavy metals to form alloys. It is readily oxidized to the +2 oxidation state, resulting in the colorless Cd^{2+} ion. Cadmium has an electronic configuration similar to that of Zn, which is an essential mineral element for living organisms. However, Cd has a greater affinity for thiol ligands than does Zn. It binds to sulfur-containing ligands more tightly than the first-row transition metals (other than Cu), but Hg and Pb both form more stable sulfur complexes than does Cd. The Cd^{2+} ion is similar to the Ca^{2+} ion in size and charge density. About two-thirds of all Cd produced is used in the plating of steel, Fe, Cu, brass, and other alloys to protect them from corrosion. Other uses include solders and electrical parts, pigments, plastics, rubber, pesticides, galvanized iron, and so on. Special uses of Cd include aircraft manufacture and semiconductors. Because Cd strongly absorbs neutrons, it is also used in the control rods in nuclear reactors. Cadmium persists in the environment and has a biological half-life of 10 to 25 years.

12.3.3 Exposure to Cadmium

12.3.3.1 Airborne Cadmium

Human exposure to Cd occurs in both occupational and general environments. Occupational exposure arises mainly from inhalation of contaminated air in some industrial workplaces. A variety of industrial activities can lead to Cd exposure. Some examples include mining and metallurgical processing, combustion of fossil fuel, textile printing, application of fertilizers and fungicides, recycling of ferrous scraps and motor oils, and disposal and incineration of Cd-containing products. Although aerial deposition is an important route of mobility for Cd, ambient air is not a significant source of Cd exposure for the majority of the U.S. population. In areas where there are no industrial facilities with Cd pollution, airborne Cd levels are around 1 ng/m^3. This indicates that, on average, an adult may inhale approximately 20 to 50 ng of Cd daily.

Tobacco smoke is one of the largest single sources of Cd exposure in humans. Tobacco in all of its forms contains appreciable amounts of the metal. Since the absorption of Cd from the lungs is much greater than from the gastrointestinal tract, smoking contributes significantly to the total body burden. Each cigarette on the average contains approximately 1.5 to 2.0 μg of Cd, 70% of which passes into the smoke.

12.3.3.2 Waterborne Cadmium

Cadmium occurs naturally in aquatic systems. Although it does not appear to be a potential hazard in open oceans, in freshwaters and estuaries accumulation of Cd at abnormally high concentrations can occur as a result of natural or anthropogenic sources. In natural freshwater, Cd usually occurs at very low concentrations (<l0 ng/L); however, the concentrations vary by area. Cd concentrations are also affected by environmental pollution; many Cd-containing wastes end up in lakes and marine water. Wastes from Pb mines, motor oils, rubber tires, and a variety of chemical industries are some examples. The amount of Cd suspended in water is determined by several factors, including pH, Cd availability, carbonate alkalinity, and concentrations of Ca and Mg. Anions such as Cl^- and SO_4^{2-} ions may complex with Cd^{2+} ions, but this possibility is small in well-oxygenated freshwater. Thus, in waters low in organic carbon and other strong complexing agents, such as aminopolycarboxylic acid, free Cd^{2+} ions predominate the dissolved species (Webb 1979).

There is a distinct difference in the forms of Cd in marine waters and freshwaters. In seawater, over 90% of the Cd is in the form of chloride salt ($CdCl_2$), while in river water Cd is present mostly as $CdCO_3$ (Nordberg 1996).

12.3.3.3 Cadmium Pollution of Soils

Cadmium pollution of soils can occur from several sources, including rainfall and dry precipitation, the deposition of municipal sewage sludge on agricultural soils, and through the use of phosphate fertilizers. In acidic soils, Cd is more mobile and less likely to become strongly adsorbed to sediment particles of minerals, clays, and sand. Cadmium adsorption depends on the concentration, pH, type of soil material, duration of contact, and the concentrations of complexing ligands.

12.3.3.4 Cadmium in Food

Cadmium exposure in the general environment comes mainly from food. Food consumption accounts for the largest source of Cd exposure by animals and humans, mainly because plants can bioaccumulate the metal (Table 12.2). Leafy vegetables, grains, and cereals often contain particularly high amounts of Cd (Table 12.3). Dietary intakes of Cd in noncontaminated areas of the world are in the range of 10 to 50 μg/day, whereas in contaminated areas the intakes may reach as high as 200 to 1,000 μg/day (Nordberg et al. 1985).

TABLE 12.2
Accumulation of Lead, Zinc, and Cadmium in Plants and Soil

	Concentration (ppm, dry weight)		
Metal	**Plants**	**Soil**	**Plant:Soil Ratio**
Pb	4.5	10	0.45
Zn	32	50	0.6
Cd	0.64	0.06	10

TABLE 12.3
Cadmium Contents in Selected Foods

Type of Food	Cd Content (μg/g wet weight)
Dairy products	0.01
Milk	0.0015–0.004
Wheat flour	0.07
Leafy vegetables	0.14
Potatoes	0.08
Garden fruits and other fruits	0.07
Sugars and adjuncts	0.04
Meat, fish, poultry	0.03
Tomatoes	0
Grain and cereal products	0.06

Aquatic organisms can potentially accumulate large amounts of Cd; therefore, animals that feed on aquatic organisms may also be exposed to the metal. Birds may be exposed to high levels of Cd as they feed on grasses and earthworms in soils treated with municipal sludge.

12.3.4 METABOLISM OF CADMIUM

Although dietary intake is the means by which humans are most highly exposed to Cd, inhalation of Cd is more dangerous than ingestion. This is because through inhalation, the organs of the body are directly and intimately exposed to the metal. Furthermore, 25% to 40% of inhaled Cd is retained, while only 5% to 10% of ingested Cd is absorbed (Figure 12.3). Following absorption, Cd appears in the blood plasma, bound with albumin (Bache et al. 1986). The bound Cd is quickly taken up by tissues, preferentially by the liver. The Cd in the liver apparently cycles, bound with metallothionein (MT), through blood, kidney, and to a small extent, bone and muscle tissue (Nordberg 1996; Bache et al. 1986). In Japanese quail fed oat grain grown on

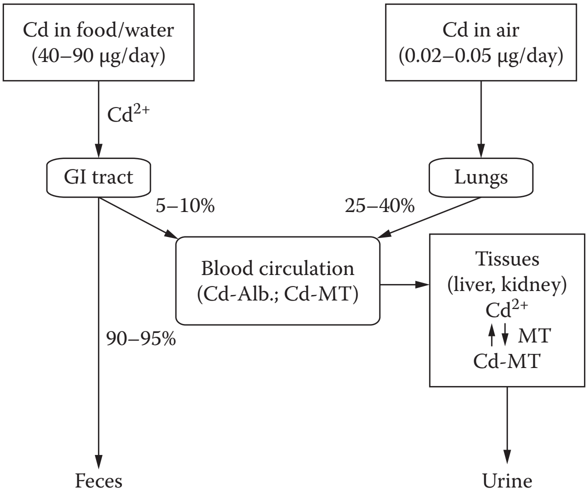

FIGURE 12.3 Cadmium metabolism in humans. Cd-alb, Cd attached to albumin; Cd-MT, Cd attached to metallothionein.

soil treated with municipal sludge, bioaccumulation was highest in the kidney, followed by liver and eggs (Friberg 1974).

Excretion of Cd in mammals seems to be minimal under normal exposure. Miniscule amounts are excreted in the feces, and an immediate 10% excretion may occur in the urine. The half-life of Cd is about 7.4 to 18 years, and the long-term excretion rate is only 0.005% per day, beginning after about 50 years of age (Bache et al. 1985; NRC 1980).

12.3.5 Cadmium Toxicity

12.3.5.1 Toxic Effects on Plants

Plant exposure to Cd occurs through air, water, and soil pollution. Cadmium is highly toxic to plants. Manifested toxicity includes stunting, chlorosis, necrosis, wilting, and depressed photosynthesis. Because of leaf surface area, leafy plants may receive large amounts of Cd from the atmosphere. However, plants are largely affected by high concentrations of Cd through waste streams coming from industrial facilities and sewage sludge as an agricultural fertilizer.

All plants can accumulate Cd, but the extent of accumulation varies with plant species and variety. Spinach, soybean, and curly cress, for instance, are sensitive to Cd, whereas cabbage and tomato are resistant. Tobacco plants have been shown to absorb high levels of Cd from the soil (Bache et al. 1985). Several factors, such as soil pH, organic matter, and cation exchange capacity, affect Cd uptake from soils. Of these factors, soil pH is the most important, with lower pH favoring the uptake. Soil organic matter and some minerals such as chloride in soil also affect Cd uptake.

In higher plants, heavy metal accumulation in the leaves is associated with a reduction in net photosynthesis. Cadmium primarily affects the photosynthetic pigments before photosynthetic function. Other studies also indicated Cd inhibition of cellular functions in plants, such as photophosphorylation, ATP synthesis, mitochondrial NADH (nicotinamide adenine dinucleotide) oxidation, and electron transport system (M.H. Yu, unpublished data, 2004).

Cadmium inhibits seed germination under laboratory conditions (Koeppe 1977; Yu 1991). Seedlings exposed to Cd solutions exhibit decreased root elongation and growth. The effect of Cd on seed germination, however, depends on several factors, including plant species. Cadmium was not found to be very toxic for germination and root growth of *Sinapis alba* seeds (Fargasova 1994), but the metal proved highly toxic to mung bean (*Vigna radiata*) seeds. For example, 1-day-old seedlings exposed to 10 and 50 μM $CdCl_2$ for 72 hours showed decreases in the fresh weight of radicles (hypocotyls and roots) by 7% and 13%, respectively. In addition, a general decrease in soluble sugar contents of the radicles occurred in the experimental seedlings. The activity of invertase, the enzyme responsible for breaking down sucrose to glucose and fructose in growing roots, was decreased by 21% and 32% in seedlings exposed to 10 and 50 μM $CdCl_2$ for 72 hours, respectively (Paert and Wikmark 1987).

12.3.5.2 Effects of Cadmium on Animals

Cadmium toxicity in animals is mostly due to the ingestion of plant matter or secondary poisoning from ingesting small prey exposed to high levels of the metal. Animals chronically exposed to Cd may exhibit emaciation, with a staggering gait, rough hide-bound skin, stringy salivation, and lacrimation. Under microscopic observations, the trachea, rumen, and spleen may show abnormal cellular structure. The trachea may show complete sloughing of its epithelium, exposing underlying submucosa. In addition, stunted epithelial lining in the bronchi and bronchioles can occur. The renal glomeruli may be shrunken due to the capillaries. In some studies, marked lymphocyte depletion in the spleen has been observed.

The toxicity of Cd to aquatic organisms is somewhat unique. In seawater, various Cd-binding ligands occur, and these appear to prevent Cd toxicity to any appreciable extent. The ligands may be derived from proteins, alginates, polyphosphates, and nucleotides resulting from tissue breakdown. In freshwaters, the liganding compounds may be provided by humic and fulvic acids from soil breakdown, citric acid, and synthetic chelating agents, often in detergents from industrial sources. The ability of these ligands to bind Cd determines Cd toxicity in aquatic systems.

Other factors affecting Cd uptake into the tissues of aquatic organisms include salinity and temperature. A decrease in salinity causes an increase in the rate of Cd uptake. The apparent reason for this is that as salinity decreases, so does the Ca concentration of the water. Calcium content of the water influences its osmolarity, which in turn affects Cd uptake. Temperature also affects Cd^{2+} absorption: When temperature increases, so does Cd^{2+} uptake (Nordberg 1996). The effects of salinity and temperature appear to be additive. The presence of some synthetic chelating agents affects the uptake of free Cd in aquatic organisms such as trout. The transfer of free Cd in chelate-free waters via fish gills is 1,000 times greater than that complexed with ethylene-diaminetetra acetic acid (EDTA) (Cooke 1981).

Because of their aquatic embryonic and larval development and their sensitivity to a wide variety of toxicants, amphibians have often been used in studying environmental contamination (Cooke 1981; Herkovits et al. 1997). In one study, the susceptibility of *Xenopus laevis* to Cd was examined during various developmental stages by exposing the embryos to varying levels of Cd, ranging from 0.1 to 10 mg Cd^{2+}/L for 24, 48, and 72 hours. Results showed that malformations occurred at all developmental stages evaluated. The most commonly observed symptoms included reduction in size, incurvated axis, underdeveloped or abnormally developed fin, and abnormally small head and eyes (Kido et al. 1989).

12.3.5.3 Effects of Cadmium on Humans

Human exposure to Cd occurs from airborne emissions, ingestion of contaminated foods, and through smoking. The adverse health effects caused by digestion or inhalation of Cd include renal tubular dysfunction from high urinary Cd excretion, high blood pressure, lung damage, and lung cancer. Cadmium and Cd compounds are “known to be human carcinogens” based on sufficient evidence of carcinogenicity in humans. This includes epidemiological and mechanistic information showing a causal relationship between exposure to Cd and Cd compounds and human cancer. In several cohort studies involving workers exposed to various Cd compounds, the risk for death from lung cancer is elevated (USDHHS 2002). A lifelong inhalation of air containing l µg Cd/m^3 is associated with lung cancer in about 2 subjects in 1,000. Long-term inhalation of $CdCl_2$ (12.5 to 50 µg/m^3) in rats showed a dose-dependent increase in the occurrence of lung cancer.

The gastrointestinal tract is the major route of Cd uptake in both humans and animals (Figure 12.3). The toxicity of the metal lies in that, after absorption, it accumulates in soft tissues as well as in the skeletal system, where it causes damage. Furthermore, Cd accumulation in animals and humans occurs throughout the life spans. For example, in humans the Cd body burden at birth is only about 1 µg; at 6 years of age, it is about 0.5 mg (500 µg), and at 64 years of age, it is about 9.6 mg (Figure 12.4). Acute Cd inhalation (>5 mg/m^3 in air), although rare, may lead to pneumonitis and pulmonary edema. Chronic exposure via inhalation, on the other hand, may cause emphysema and chronic pulmonary effects. The sites of most Cd accumulation are the liver and kidney. After inhalation or gastrointestinal absorption, Cd is concentrated in the kidney, where its half-life may exceed 10 to 20 years. One of the most widely known toxic effects manifested by Cd poisoning is nephrotoxicity. Although acute Cd exposure through ingestion of food contaminated with high levels

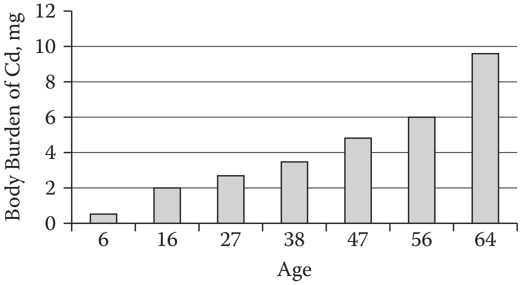

FIGURE 12.4 Cadmium accumulation in humans at different ages.

of the metal can lead to proteinuria, this is rather rare. More commonly, adverse renal effects are seen with exposure to low levels of Cd. The effects are manifested by excretion of low molecular weight plasma proteins such as β_2-microglobulin and retinol-binding protein (RBP).

The widely reported Cd poisoning episode itai-itai-byo or ouch-ouch disease occurred in Japan after World War II. The disease was caused mainly by ingestion of Cd-contaminated rice produced from rice paddies that received irrigation water contaminated with high levels of the metal. Subsequent studies showed that persons with low intake of Ca and vitamin D were at a particularly high risk (Nriagu 1980).

According to Nordberg (1985), the mechanisms involved in tubular Cd nephrotoxicity may include the following: It is assumed that the rate of influx of Cd-metallothionein (Cd-MT) into the renal tubular cell compartment on the one hand and the rate of de novo synthesis of MT in this compartment on the other hand regulate the pool of intracellular “free” Cd ions that can interact with cellular membrane targets in the tubules. When there is efficient MT synthesis and influx of Cd-MT into the lysosomes is limited, the free Cd pool is limited, and no membrane damage will occur.

Cd impairs many plant cellular functions, including ATP synthesis, succinate oxidation, photophosphorylation, mitochondrial NADH oxidation, and electron transport (Rauser 1990). Cd is a potent enzyme inhibitor, affecting a variety of plant enzymes, such as phosphoenolpyruvate carboxykinase, lipase, and invertase.

In humans and animals, Cd inhibits alkaline phosphatase and adenosine triphosphatase (ATPase) of myosin and pulmonary alveolar macrophage cells. Cd appears capable of inhibiting phase I and phase II xenobiotic biotransformation (Chapter 4) in the liver and kidney of rainbow trout. Hemoglobin concentrations in fish exposed to Cd are decreased, leading to anemia and liver damage. Inhibition of protein synthesis and enzyme activity and competition with other metals are other deleterious effects of Cd on aquatic organisms (Nordberg 1996; Friberg 1974).

Two mechanisms appear to be involved in enzyme inhibition by Cd; one is through binding to SH groups on the enzyme molecule, as is the case with Pb and Hg, and another is through competing with Zn and displacing it from metalloenzymes. Like other heavy metals of concern, Cd can also bind with SH-containing ligands in the membrane and other cell constituents, causing structural and functional disruptions. For instance, by inducing damage to mitochondria, Cd can uncouple oxidative phosphorylation and impair cellular energy metabolism. Induction of peroxidase activity by Cd in tissues of *Oryza sativa* suggests Cd-dependent lipid peroxidation, resulting in membrane damage. As discussed in Chapter 4, membrane damage due to lipid peroxidation is mediated by oxygen radicals and induction of peroxidase, superoxide dismutase (SOD), and catalase.

Interest in the defense response of living organisms acutely exposed to Cd is growing. Plants, algae, and bacteria respond to heavy metal toxicity by inducing different enzymes, creating ion influx/efflux for ionic balance, and synthesizing small peptides. These peptides bind metal ions and reduce toxicity. Certain plant species exposed to Cd and some other heavy metals produce a class of sulfur-rich polypeptides termed phytochelatins to complex and thus neutralize the metals. According to Rauser (Reddy and Prasad 1990), phytochelatins act by directly binding to metal ions through chelation to form mercaptide complexes. Reddy and Prasad (Hamilton and Valberg 1974), for instance, observed formation of a callus in plants exposed to Cd. The plants had higher protein content than the control plants. Over 200 plant species have been found capable of forming phytochelatins.

12.3.6 Cadmium and Nutrition

A close relationship exists between Cd toxicity and nutrition. For example, at moderate levels, Cd can antagonize several essential metals such as Zn, Cu, selenium (Se), and Fe. The effect of Cd on mammals is thus influenced by the relative intakes of these and other metals by the animals and vice versa. Cd causes the serum Zn content to decrease and adversely affect serum insulin levels and glucose tolerance. This last effect can be prevented in rats by increased Zn intake (Book et al. 1973).

Iron deficiency can influence Cd toxicity. Cd uptake by the body is increased during Fe deficiency or anemia. In mice, Cd has also been shown to compete with Fe in their transport system. Studies on Fe absorption in mice receiving Cd in their drinking water showed that Fe absorption was significantly inhibited at a Cd dose of 1 mg/mL (Maji and Yoshida 1974).

The effect shown in laboratory mice has also been observed in humans. Mild anemia commonly occurs among industrial workers exposed to Cd dust fumes. Concern is growing over the general populations exposed to Cd as levels of Cd in the environment, particularly in highly industrialized areas, have increased over the last several decades. As mentioned previously, Cd, once absorbed, is not readily excreted. With a long biological half-life in humans, it is possible that the concentrations of Cd may eventually become high enough to inhibit Fe absorption. Such possibility is of particular concern because Fe deficiency is one of the most common nutritional problems in the world.

Newborn and young animals have the greatest increase in Cd absorption rate of all ages. The mechanism for this appears to be related to the absorption of Cd through milk. Because young animals need Ca for their growth and development, high amounts of Ca-binding protein (CaBP) are produced. It is thought that Cd utilizes the same transport system as Ca, or at least inhibits the latter's functioning. The effect of Cd on the CNS is attributed to displacement of Ca from its action sites in the neuromuscular junction by Cd (Webb 1979). Dietary protein is also related to the toxicity of ingested Cd. A low-protein diet may lead to increased absorption of Cd and thus increased toxicity. MT synthesis is decreased under low-protein conditions. A low-protein diet may lower MT availability for binding free Cd, resulting in increased Cd toxicity. Cd has also been shown to be related to lipid peroxidation and decreased phospholipid content in rat brains (Maji and Yoshida 1974). Such lesions may partly account for the observed Cd-induced neurotoxicity.

Another nutrient with an important role in Cd toxicity is vitamin C (ascorbic acid). Fox and Fry (1970) showed that vitamin C and Fe supplementation markedly reduced Cd accumulation in various soft tissues of rats, resulting in lowered toxicity. It is believed that vitamin C enhances Fe absorption through reduction of Fe^{3+} to Fe^{2+} as well as through chelation with Fe^{3+}. However, it is possible that other mechanisms may also be involved. One of the possible mechanisms is the reduction of lipid peroxidation caused by vitamin C. As noted previously, Cd causes membrane damage, possibly through induction of lipid peroxidation. Together with vitamin E, vitamin C could reduce Cd-induced damage by reacting with lipid peroxy radicals, thus limiting lipid peroxidation.

12.4 MERCURY

12.4.1 Introduction

Mercury is the only common metal that is liquid at room temperature. It has a high specific gravity, 13.6 times that of water. Its boiling point is 357°C, which is relatively low. Mercury has a long liquid range of 396°C, and it expands uniformly over this range. This linear expansion, in addition to the fact that Hg does not wet glass, makes the metal useful in thermometers. Mercury has the highest volatility of any metal. Its good electrical conductivity makes it exceptionally useful in electrical switches and sealed relays. Many metals dissolve in Hg to form amalgams (alloys).

Mercury is rare in the earth's crust (0.1 to 1 ppm) and is not widely distributed, but it is ubiquitous, being measurable in trace amounts in most foods and water. Mercury has no known biological role and is an industrial health hazard because of its diverse use. It is a bioaccumulative metal that is fat soluble and has many damaging effects on living organisms.

12.4.2 Extraction and Uses of Mercury

Although several forms of ore occur, the principal one is *cinnabar,* the red sulfide, HgS. The extraction of Hg from the sulfide ore is accomplished by roasting the ore in air or with lime, as follows:

$$\text{Roasting in air: } HgS + O_2 \rightarrow Hg + SO_2 \tag{12.2}$$

$$\text{Roasting with lime: } 4HgS + 4CaO \rightarrow 4Hg + 3CaS + CaSO_4 \tag{12.3}$$

The resultant metal is condensed from the furnace gases.

While Hg has a long history of use among preindustrial humans, it is also used extensively by modern industry, such as in the manufacture of Hg batteries and other electrical apparatus. Science employs it in laboratory equipment, and it is widely used in barometers. Many Hg compounds, particularly acetate, oxide, chloride, sulfate, and phosphate, are used as catalysts in industrial chemistry. Mercury compounds are added to paints as preservatives. In addition, Hg is used in jewelry making, pesticides, and other manufacturing processes. The light emitted by electrical discharge through Hg vapor is rich in ultraviolet (UV) rays, and lamps of this kind in fused quartz envelops are widely used as sources of UV light, such as in UV spectrophotometers. High-pressure Hg vapor lamps are now widely used for street and highway lighting.

In the United States, the largest user of Hg is the chlor-alkali industry, in which chlorine and caustic soda are produced by electrolysis of salt (NaCl) solution. In one technical method of producing chlorine, a flowing Hg cathode is used. The Na^+ ions discharge at the Hg surface, forming sodium amalgam. The resultant amalgam is continuously drained away, and as it is treated with water, NaOH solution and Hg are produced:

$$\text{Hg-Na} \xrightarrow{H_2O} \text{NaOH (solution)} + \text{Hg} \tag{12.4}$$

12.4.3 Sources of Mercury Pollution

Mercury is a naturally occurring metal dispersed throughout the ecosystem. Mercury contamination of the environment is caused by both natural and anthropogenic sources. Natural sources include volcanic action, erosion of Hg-containing sediments, and gaseous emissions from Earth's crust. The majority of Hg comes from anthropogenic sources. Mining, combustion of fossil fuels in municipalities and hospitals (e.g., Hg content of coal is about 1 ppm), transporting Hg ores, processing pulp and paper, incineration, use of Hg compounds as seed dressings in agriculture, and exhaust from metal smelters are some examples. In addition, Hg waste is found as a by-product of chlorine manufacturing plants and gold recovery processes and is found in used batteries and lightbulbs.

Gold mining in the Amazon in recent years has led to Hg pollution. Hg enters the environment during each of the two steps involved in acquiring the gold. First, the sediments are taken from river bottoms and land mining sites and forced through sieves. The sieves are coated with mercury, which bonds with the gold, separating it from the rest of the material. A large amount of Hg remains in the leftover soil and is a threat to the environment when this soil is discarded. Second, the gold-mercury amalgam is heated to purify the gold by vaporizing the Hg. When carried out in an unsealed container, Hg vapor will be emitted into the environment. The Hg evaporated or burned in these operations can travel long distances, with subsequent precipitation by tropical rainstorms, leading to water pollution. As rainwater is rich in Hg^{2+} species formed by oxidation of Hg gas, pollution of fish can occur even in remote areas.

This practice releases elemental Hg to the atmosphere, exposing people nearby to toxic fumes. According to UNEP (United Nations Environment Programme), about 1000 metric tons of Hg are released per year, accounting for about a third of all Hg release to the environment from human activities. Use of Hg in small-scale mining appears to be highest in China, which releases 200 to 250 metric tons into the atmosphere per year. Indonesia comes in second, with 100 to 150 metric tons of release. Brazil, Bolivia, Colombia, Ecuador, Ghana, Peru, the Philippines, Venezuela, Tanzania, and Zimbabwe each emit 10 to 30 metric tons per year.

12.4.4 Biotransformation of Mercury

Various forms of Hg are present in the environment. Conversion of one form to another occurs in sediment, water, and ash (Murozami et al. 1969) and is catalyzed by various biological systems. For example, following its release and being washed back down to earth in rainwater, Hg often finds its way to eventual deposition in lakes and seas. Microorganisms then convert the elemental Hg into methylmercury

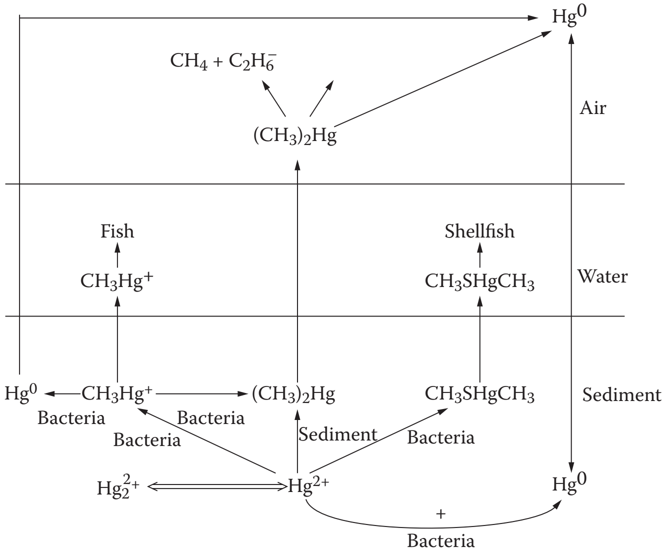
Hg0
CH4 + C2H6
(CH3)2Hg
Air
Fish
CH3Hg+
Shellfish
CH3SHgCH3
Water
Hg0
CH3Hg+
(CH3)2Hg
CH3SHgCH3
Sediment
Bacteria
Bacteria
Bacteria
Sediment
Bacteria
Hg2 2+
Hg2+
Hg0
+
Bacteria

FIGURE 12.5 The mercury cycle showing bioaccumulation of mercury in fish and shellfish. (*Source*: National Research Council. 1978. *An assessment of mercury in the environment.* National Academy Press/NAS, Washington, DC.)

(MeHg) through a process called methylation. The MeHg thus formed may then begin its march up the aquatic food chain. Or, it may be split in a reaction mediated by bacteria, as shown in Figure 12.5.

12.4.4.1 Biomethylation of Mercury

Soluble inorganic mercury salts can be converted to MeHg and dimethylmercury, $(CH_3)_2Hg$. This reaction can occur both aerobically and anaerobically. Alkyl cobalamines serve as alkylating agents, while methyl-B_{12} acts as a coenzyme in the reaction:

$$Hg^{2+} + 2RCH_3 \rightarrow (CH_3)_2Hg \rightarrow CH_3Hg^+ \tag{12.5}$$

12.4.4.2 Demethylation of Methylmercury

The methyl group in $(CH_3)_2Hg$ may be split off to give rise to Hg^{2+} ion and methane and ethane. The reaction is called demethylation and is catalyzed by two enzymes: a hydrolase and a reductase. The hydrolase hydrolyzes the mercury-carbon bond, yielding Hg^{2+} ions and methane. The reductase reduces the Hg^{2+} ion to metallic Hg. The Hg^{2+} ion may eventually be volatilized from the aqueous medium into the atmosphere. Demethylation appears to be much slower than methylation.

12.4.4.3 Methylmercury Biosynthesis and Diffusion into Cells

The rate of MeHg synthesis is determined by the microbial community and concentrations of soluble mercuric or mercurous species and methyl-B_{12} (which acts as a coenzyme). The bioaccumulation of MeHg into the tissues of higher organisms, such as fish, appears to be controlled by diffusion. For example, MeHg-chloride diffuses through cell membranes into cells in 20×10^{-9} seconds. Once MeHg diffuses through the cell membrane, it is bound by –SH groups, thus maintaining the concentration gradient across the membrane, eventually leading to bioaccumulation. The mercury cycle demonstrating the bioaccumulation of Hg in fish and shellfish is depicted in Figure 12.5.

12.4.5 TOXICITY OF MERCURY

12.4.5.1 Effects of Mercury on Algae

Very low concentrations of Hg can be lethal to some species of algae and impair the growth of others. Organomercurials were shown to retard the growth and viability of several species of marine algae more effectively than inorganic Hg (Matida et al. 1971). Concentrations as low as 0.1 ng/L of several alkylmercurial fungicides have been shown to decrease the growth and photosynthesis of some freshwater phytoplankton. The high sensitivity of phytoplankton to Hg compounds may be due to the high lipid content in the membranes or to the inhibition of lipid synthesis by the metal. Because phytoplankton is situated at the lowest trophic level in the aquatic ecosystem, accumulation of Hg in phytoplankton can lead not only to disruption of the food chain but also to bioaccumulation of the metal in organisms of higher trophic levels.

12.4.5.2 Effects of Mercury on Plants

All plants appear to accumulate traces of Hg. Total Hg levels in most common edible plants and foods derived from plants range from less than 1.0 to 300 ng/g. The concentration of Hg in plants depends on Hg deposits in the soil, locality, plant species, the chemical form of the Hg, and soil aeration. Some plants have a barrier to the uptake and circulation of inorganic Hg salts and organically complexed mercurials adsorbed on clay, while others have no barrier against the uptake of gaseous Hg through the roots. In soils where decaying sulfides release gaseous elemental Hg, the vegetation contains 0.2 to 10 ng/g (on a dry weight basis).

Like Pb and Cd, Hg can cause deleterious effects on different species of plants. Hg is particularly toxic to barley plants, more so than Pb, Cr, Cd, Ni, and Zn (Oberlander and Roth 1978). In rapidly dividing onion root cells, MeHg at 2.5×10^{-7} *M* interferes with normal chromosome segregation by disrupting the mitotic spindle function (Ramel 1967). Hg also impairs germination, as shown by depressed root elongation and shoot growth (Yu et al. 1999).

Greger and Dabrowska (2010) studied whether MeHg uptake and the MeHg formation in the shoots of water spinach (*Ipomoea aquatic*) were affected by the presence of a high nutrient level in the growth medium and found that the nutrient level did not influence MeHg uptake. But, high nutrient levels reduced the distribution of MeHg to the shoots 2.7-fold versus low nutrient.

12.4.5.3 Effects of Mercury on Animals

Freshwater and marine organisms and their predators normally contain more Hg than terrestrial animals. Levels in top predatory fish are higher. Fish may accumulate

Hg in excess of the 0.5 mg/g Food and Drug Administration (FDA) guideline. This accumulation is part of a dynamic process in which an organism strives to maintain equilibrium between intake and excretion. Numerous analyses have indicated that much of the tissue Hg in most fish is in the form of MeHg (Westoo 1973). The mercury accumulated in fish comes primarily from absorption of the water across the gill or through the food chain, although some higher species may convert inorganic Hg into MeHg. Some Hg is also taken up through the mucous layer or skin.

The metabolic rate of the fish and the Hg concentration in the aquatic ecosystem appear to be more important factors in bioaccumulation than age or exposure rate. Since increased temperature enhances metabolic rate, more Hg is concentrated in the summer than in the winter. The toxicity of Hg and other heavy metals to fish increases with an increase in temperature. The 96-hour LC_{50} (concentration that kills 50%) of Hg for freshwater crayfish, *Procambarus clarkii* (*Girarcfy*), was found to be 0.79 mg/L at 20°C, 0.35 mg/L at 24°C, and 0.14 mg/L at 28°C (Del Ramo et al. 1987).

Wild birds concentrate the highest levels of Hg in the kidney and liver, with less in the muscle tissues. Swedish ornithologists observed the first Hg-related ecological problems in the 1950s. Many species of birds declined in both numbers and breeding success, while Hg levels increased in the feathers of several species of seed-eating birds. In the United States and Canada, elevated levels of Hg were also found in seed-eating birds and their predators, presumably through eating Hg-treated seed dressings. In 1970, both countries banned alkylmercurial seed dressings, and the levels decreased in game birds that do not feed on aquatic organisms.

Age and diet markedly influence the rate of Hg absorption in animals. Suckling mammals have a high intestinal absorption rate due to their milk diet. Whole-body retention, high blood levels, and high accumulation in various organs, such as the brain, are seen in sucklings when compared with adult animals. For example, the absorption rate (as percentage of oral dose) of ^{203}Hg in 1-week-old suckling rats was 38.2%, whereas in 18-week-old rats on either a milk diet or a standard diet, the rate was 6.7% and 1%, respectively (Stara and Kello 1979).

The neurotoxicity of MeHg varies greatly between animal species. For example, nonhuman primates and cats metabolize MeHg similarly to humans, but rats or mice rapidly metabolize the compound to a less-toxic inorganic form (Task Group on Metal Toxicity 1976).

12.4.5.4 Effects of Mercury on Human Health

There is an enormous difference in toxicity between chemically different forms of Hg. MeHg and $(CH_3)_2Hg$ are exceedingly poisonous, and low concentrations are still very dangerous, while other forms might not be dangerous at all, such as dental amalgam. Mercury vapor is quite poisonous. Mercury is volatile enough that its vapor pressure at room temperature would produce very harmful levels of Hg vapor at equilibrium. But, the vapor is extremely heavy and would stay close to the floor. Also, the diffusion of Hg into air is very slow because of its high atomic weight. Spilled Hg might produce Hg vapor, but high levels would be very close to the ground. Unless one lay down and breathed air at ground level, the danger to any person would be small.

Almost all the MeHg in the human diet appears to come from fish or other seafood and possibly from red meat. Until recently, the Hg present in either the atmosphere or drinking water supplies was not considered to contribute significantly to the MeHg burden in the human body. However, according to EPA risk assessment of human health, Hg is the toxin of greatest concern among 188 air toxics emitted from power plants. Coal-fired power plants are the largest source of anthropogenic Hg air emissions in the United States (40% of total emissions) (Schnoor 2004). Some researchers consider a plausible link between anthropogenic releases of Hg from industrial and combustion sources and MeHg in fish. In the United States, among women of childbearing age, 7.8% had blood levels of Hg exceeding the reference dose, the level at which most people could be exposed without risk.

The two major Japanese outbreaks of MeHg poisoning in Minamata Bay and in Niigata were caused by industrial discharge of MeHg and other Hg compounds into Minamata Bay and into the Agano River, resulting in accumulation of MeHg in fish and shellfish. The median total Hg level in fish caught in Minamata Bay at the time of the epidemic was estimated at 11 mg/g fresh weight. More than 700 cases of MeHg poisoning were identified in Minamata and more than 500 in Niigata (World Health Organization [WHO] 1976). (The Minamata Bay episode was caused by a chemical plant, which was manufacturing acetaldehyde using mercuric sulfate as a catalyst and discharging the waste containing high levels of Hg into the bay. Following the incident, the Chisso Corporation, then with 7,000 employees, went bankrupt. The sediments contaminated with Hg were dredged, put into large steel drums, sealed, and buried at the bottom of the bay. Clean soils were then brought to cover about 60% of the bay, converting it into a flat area of about 2×10^6 m^2. The cost for the project totaled about $300 million.)

The critical organ concentration of MeHg may differ for different stages of the human life cycle. The developing fetal and newborn brain may be the most sensitive organ (i.e., critical organ) in terms of human MeHg toxicity. During the Japanese Minamata outbreak, 23 infants with severe psychomotor signs of brain damage were diagnosed. They were born to mothers who had consumed fish taken from the bay. The mothers were reported to have no symptoms or signs of MeHg poisoning other than mild paresthesia (an abnormal sensation, such as prickling, itching, etc.). It was concluded that MeHg had crossed the placenta, and that the fetal brain was much more sensitive than the adult brain.

The largest outbreak of MeHg poisoning ever recorded occurred in Iraq during 1971 to 1972. The poisoning resulted from consumption of bread made from wheat that had been treated with a MeHg fungicide. It was reported that more than 6,000 children and adults were poisoned, with nearly 500 deaths. Symptoms observed among the victims included paresthesis, ataxia, dysarthria, and deafness (NRC 1978). In this outbreak of MeHg poisoning, an infant's blood Hg level was found to be higher than the mother's during the first few months of life, supporting the suggestion that the fetal brain is the critical organ in the exposed pregnant female.

The relative toxicity of various Hg compounds toward tissue depends on the relative ease of their formation of the Hg^{2+} ion. $HgCl_2$ is most toxic, while some nonionizable organic mercurials are relatively safe. Arylorganic Hg causes skin burns at high concentrations, while at low concentrations it may cause irritative dermatitis, but alkylorganic Hg is most likely to accumulate in nervous tissue.

Inhalation of Hg vapor is perhaps the greatest source of danger in industrial and research laboratories. Hg vapor can diffuse through the alveolar membrane and reach the brain, where it may interfere with coordination. Pronounced brain damage occurs in victims of Hg poisoning.

Chronic Hg poisoning may result from exposure to small amounts of Hg over long periods, such as may occur in industries that use Hg or its salts. The symptoms include salivation, loss of appetite, anemia, gingivitis, excessive irritation of tissues, nutritional disturbances, and renal damage accompanied by proteinuria. Acute Hg poisoning results from ingestion of soluble Hg salts. Mercury chloride precipitates all proteins with which it comes into contact. Vomiting usually occurs a few minutes after ingestion. The victim experiences abdominal pain. Loss of fluids and electrolytes occurs. The biological half-life of Hg is estimated to be 70 days. The critical daily intake has been estimated to be 300 mg Hg as MeHg for an average 70-kg man.

Chemists and biologists across the United States were shocked in the summer of 1997 by the death of Dartmouth College chemistry professor Karen E. Wetterhahn as a result of acute exposure to dimethylmercury ($[CH_3]_2Hg$) (Blayney et al. 1997). It was reported that she was apparently transferring the compound in a fume hood when 0.1 to 0.5 mL of it spilled on the disposable latex gloves she was wearing and permeated them, quickly seeping into her skin. She became ill a few months later and died of Hg poisoning less than a year after the exposure.

According to the FDA, the dietary Hg guidelines were established to limit consumers' MeHg exposure to levels 10 times lower than the lowest levels associated with adverse effects. Americans who consume twice as much Hg as the FDA recommends are still protected by a 500% cushion. The same generous safety margin applies to the EPA's mercury "reference dose" (see Table 12.4).

The U.S. government Institute of Medicine (a division of the National Academies of Science) warned in a major 2006 report that a "spillover effect" from one-size-fits-all fish warnings could deny most consumers the health benefits of seafood consumption (Table 12.4). This report demonstrated a severe disagreement between serious scientists and activists. There are no scientifically documented cases of Americans developing Hg poisoning from eating commercially available fish. The only documented cases in the medical literature are from Japan in the 1950s and 1960s (cf. the Minamata Bay episode).

Some additional materials concerning the health effects of fish consumption are presented in Table 12.4.

12.4.6 BIOLOGICAL EFFECTS

Mercury, like many other heavy metals, is extremely toxic because, as an ion or in certain compounds, it is soluble in water. For this reason, it is readily absorbed into the body, where it tends to combine with and inhibit the functioning of various enzymes. The ultimate effects of Hg in the body are similar to those of Pb and Cd: inhibition of enzyme activity and cell damage. Hg has been reported to inhibit a large number of enzyme systems (Boyer et al. 1959). The particular reactivity of Hg

TABLE 12.4
Essential Mercury Facts

1 According to the Food and Drug Administration (FDA), its dietary Hg guidelines were "established to limit consumers' methyl mercury exposure to levels 10 times lower than the lowest levels associated with adverse effects." Americans who consume twice as much Hg as the FDA recommends are still protected by a 500% cushion. The same generous safety margin applies to the EPA's mercury "reference dose."

2 The U.S. government Institute of Medicine (a division of the National Academies of Science) warned in a major 2006 report that a "spill-over effect" from one-size-fits-all fish warnings could deny most consumers the health benefits of seafood consumption. This report demonstrated a severe disagreement between serious scientists and activists.

3 There are no scientifically documented cases of Americans developing Hg poisoning from eating commercially available fish. The only documented cases in the medical literature are from Japan in the 1950s and 1960s (cf. the Minamata Bay episode).

4 The federal government's mercury-in-fish recommendations are based largely on a single study whose participants were exposed to Hg by eating whale meat—not fish. (The study was conducted in Denmark's Faroe Islands. Unlike fish, whale meat is contaminated with a variety of pollutants, so isolating mercury's effects is practically impossible. In 2004 the lead Faroe researcher acknowledged in *The Boston Herald* that "fish consumption does not harm Faroese children. … The fish consumption most likely is beneficial to their health.")

5 A 12-year study conducted in the Seychelles Islands (in the Indian Ocean) recently found no negative health effects from exposure to Hg through heavy fish consumption. (On average, people in the Seychelles eat between 12 and 14 fish meals every week, and the Hg levels measured in the island natives are higher than those measured in the United States. But, they suffered no ill effects from Hg in fish, and they received a significant health benefit from eating fish in the first place.)

6 In February 2007, the *Lancet* (the United Kingdom's most prestigious medical journal) published U.S. government-funded research demonstrating a clear health benefit to children whose mothers ate large amounts of fish while pregnant. Researchers wrote that they could find "no evidence to lend support to the warnings of the U.S. advisory that pregnant women should limit their seafood consumption." Of the more than 9,000 pregnant women in this study, those who ate the most fish—regardless of Hg levels—had children with the highest IQs.

7 Studies published in 2005 in the *American Journal of Preventive Medicine* found that even eating small amounts of fish each week can result in a 17% lower risk of heart disease, a 12% lower risk of stroke, and (when eaten by pregnant women) a modest increase in children's IQ. The omega-3 fats found in fish can also protect against Alzheimer's disease, arthritis, breast and prostate cancer, and many other conditions.

8 Researchers at Harvard University concluded that the health benefits of fish "greatly outweigh the risks," including those from trace amounts of Hg. (Their study was published in *JAMA* in October 2006.)

9 Over 40 years of scientific research has established that Se, a plentiful nutrient in fish, can effectively neutralize the toxicity of trace amounts of Hg in seafood. According to the USDA, 16 of the 25 best sources of dietary Se are ocean fish.

TABLE 12.4 (*Continued*)
Essential Mercury Facts

10	There is solid scientific evidence that the amount of Hg in fish has remained the same (or even *decreased*) during the past century. Researchers from Princeton University, Duke University, and the Los Angeles County Natural History Museum have all compared specimens of ocean fish preserved between 25 and 120 years ago with current samples of the same species. In these studies, Hg levels in the fish stayed the same or declined.

Source: After http://mercuryfacts.org/.

with thiol ligands has further confirmed the selective affinity of this metal to react with the –SH group, as shown in the following example with MeHg:

$$\mathrm{RSH} + \mathrm{CH_3Hg^+} \rightarrow \mathrm{R{-}S{-}Hg{-}CH_3} + \mathrm{H^+} \tag{12.6}$$

Mercury is known to affect the metabolism of mineral elements, such as sodium (Na) and potassium (K), by increasing their permeability. Hg also

- inhibits the active transport mechanism through dissipation of normal cation gradient
- destroys mitochondrial apparatus
- causes swelling of cells, leading to lysis
- decreases α- and γ-globulins while increasing β-globulin, suggesting liver dysfunction
- decreases DNA content in cells and adversely affects chromosomes and mitosis, leading to mutagenesis

Exposure of rat lung cultures to low concentrations of $\mathrm{Hg^{2+}}$ ions (added as $\mathrm{HgCl_2}$) appeared to be cytotoxic as it altered the rates of DNA, RNA, and collagen synthesis. For example, exposure to 0.1 to 10.0 μ*M* $\mathrm{Hg^{2+}}$ ions increased DNA synthesis by 2.5 to 3.5 times after 24 hours, but the rate decreased over the 5-day culture period (Bhatnagar et al. 1979).

The MT protein receptor present in kidney tissue tends to bind actively with Hg and may thus exercise a protective effect. When the MT receptors are saturated with Hg, morphologic damage becomes manifest. An adaptive mechanism may exist; MT content in the kidneys increases with repeated Hg exposure.

A number of researchers have indicated the association between iodine and mercury. According to Clinch (2009), iodine increases urinary excretion of mercury.

12.4.7 MERCURY AND NUTRITION

The interaction of MeHg with –SH groups is considered the natural biological sink for the Hg compound. Approximately 95% of the Hg bound to fish protein has been shown to be part of the MeHg–cysteinyl coordination complex. The selenohydryl group was shown to bind MeHg 100 times more tightly than the –SH group (Sugiura et al. 1976). In addition to Se, vitamin E is known to protect against the toxic effect of MeHg. However, a much higher concentration of this vitamin is required to provide the same level of protection as with Se.

Earlier research showed that ascorbic acid added in the diet overcame growth depression caused by 500 ppm Hg in chicks. It was shown that there was an effect of ascorbic acid on Hg metabolism that was not mediated through Fe metabolism because adding 1,000 ppm Fe in the diet did not overcome the growth depression caused by Hg. High dietary levels of ascorbic acid might result in increased urinary excretion of cations to balance the excretion of the ascorbate anion and thus increase the rate of excretion of toxic elements (Yu 2005).

Indeed, Hg in fish has been shown to be widespread. A federal study of Hg contamination released in August 2009 found the toxic substance in every fish tested at nearly 300 streams across the country, a finding that underscores how widespread Hg pollution has become (U.S. Geological Survey 2009).

In the United States, fish consumption has increased considerably (ca. 25%) over the last decade. This increase in fish consumption is mainly attributed to general knowledge about its nutritional value, including, for example, high protein content, and relatively low levels of calories, cholesterol, fats, particularly saturated fats, while remaining rich in omega-3 fatty acids. Some researchers, however, are concerned about the trend of increased fish consumption because they fear that increased seafood consumption could mean increased risk of exposure to highly toxic MeHg. Recent studies showed that such fears are not well supported (see Table 12.4).

The study by the U.S. Geological Survey is the most comprehensive look to date at Hg in the nation's streams. From 1998 to 2005, scientists collected and tested more than a thousand fish from 291 streams nationwide. While all fish had traces of Hg contamination, only about a quarter had levels exceeding what the EPA indicated is safe for people eating average amounts of fish (Chau and Kulikovsky-Cordeiro 1995).

Dietary Se has been shown to exhibit a protective effect against Hg toxicity (Hanzlik 1981). Treatment with Se reduced the lethal and neurotoxic effects of MeHg and other Hg compounds. Although the protective effect of Se in Hg toxicity has been known for some time, the exact mechanism involved has not been clear.

Hg toxicity is typically associated with the element's high affinity for binding sulfur in cysteine (an amino acid) residues of proteins and enzymes. A second toxicity mechanism involves mercury's interaction with Se, an important antioxidant element in humans. Hg is known to reduce the bioavailability of Se by forming insoluble mercury selenide species and by binding to active sites of selenoenzymes (Nieboer and Nriagu 1992).

12.5 NICKEL

12.5.1 INTRODUCTION

Nickel (Ni) is a white metal, with a faint tinge of yellow. Although it is the fifth-most-abundant element in the biosphere, Ni was only discovered through the mining of other metals. Its principal ores are *nickelite,* NiAs; *millerite,* NiS; and *pentlandite,* (Ni,Fe)S. Nickel is quite mobile through air, water, and soil. Historically, the focus of concern for this metal was how to increase worker safety, but many researchers are now paying more attention to examining nickel's role in the health of ecosystems.

Nickel was largely ignored for industrial use until just before 1900, when the Mond carbonyl process was discovered as a way to remove the metal in a pure form from the mined ores. This process was the key to triggering concern for worker safety because part of it involved nickel carbonyl ($Ni[CO]_4$) gas, the most toxic form of the metal. Other forms of Ni, however, play an uncertain role in the safety of workers and the public. Overall demand for Ni has been increasing over time, mostly due to increasing stainless steel production. Nickel, including the forms of nickel carbonate, is used in approximately 250,000 industrial applications.

12.5.2 SOURCES OF ENVIRONMENTAL NICKEL POLLUTION

Environmental contamination by Ni occurs naturally and anthropogenically. The natural sources include volcanoes, ocean spray, soil dust, and forest fires, with a particulate size ranging from 2 to 10 μm. Examples of anthropogenic sources are the mining, smelting, and refining of Ni, with release of much smaller size particulate matter (0.1 to 2.0 μm). In scrap recycling, Ni is released from the melting of stainless steel. Other mining, including gold mining, can release Ni into the surrounding environment as a by-product, usually with other leachates. Nickel sulfate is released in the burning of fossil fuels and sewage incineration.

Nickel air pollution includes, in addition to those mentioned, the processing of Ni, burning of petroleum products, and plastic production. The concentrations of Ni in air are increased over industrialized areas. For example, the highest U.S. concentration of the metal is found in South Carolina, with 116 ng of Ni/m^3 of air (Nriagu 1980).

Nickel-cadmium batteries are a potential source of Ni water pollution resulting from leaching from waste sites. In addition, an elevated Ni concentration is often found in the water of lakes near industrial areas.

12.5.3 HEALTH EFFECT

The most common type of exposure to the public is through direct skin contact with Ni plating. Nickel carbonyl gas, the most toxic of Ni compounds, was the earliest to cause deaths in refineries. In April 1953, the Gulf Oil Company in Port Arthur, Texas, exposed more than 100 workers to nickel carbonyl gas during repair work. Two workers died at the scene, and 31 were hospitalized. Some of the immediate symptoms included headaches, nausea, weakness, dizziness, vomiting, and epigastric pain. There was a latency period of 1 to 5 days, followed by secondary symptoms, including chest constriction, chills and sweating, shortness of breath, coughing, muscle pains, fatigue, gastrointestinal discomfort, and in severe cases, some convulsions and delirium.

Nickel carbonyl is a volatile liquid with extraordinary toxicity, particularly to the lungs. It is an intermediate produced in refining nickel ore. The mechanism of this toxicity is not known, but the lungs play a major role in both absorption of $Ni(CO)_4$ vapors and excretion of parenterally administered $Ni(CO)_4$. Thus, 60 minutes after intravenous administration of ^{14}C- or ^{63}Ni-labeled $Ni(CO)_4$, 25% was exhaled unchanged, 11% was exhaled as ^{14}CO, 10% was present as ^{14}C carbon-monoxyhemoglobin, and 6.5% was present as unchanged $Ni(CO)_4$. Translocation of ^{63}Ni from erythrocytes to plasma was correlated with the disappearance of $Ni(CO)_4$ from whole blood.

Since metal-CO bonds are greatly weakened by oxidation of the central metal, it is conceivable that $Ni(CO)_4$, like Hg, might be biotransformed in vivo by the catalase-H_2O_2 system, as outlined in Equation 12.7 (Nieboer and Nriagu 1992).

$$\mathrm{Ni(CO)_4} \xrightarrow[\text{(enzyme)}]{-2e} [\mathrm{Ni(CO)_4^{2+}}] \xrightarrow[\text{fast}]{} 4\mathrm{CO} + \mathrm{Ni^{2+}} \tag{12.7}$$

Worker exposure also occurs through inhalation of Ni dust formed in the refining process, through grinding, calcination, and leaching of the metal ore. This exposure, especially to insoluble forms of Ni such as nickel oxides, nickel subsulfide, and metallic nickel, has been hypothesized as a possible carcinogen that prompts carcinomas 20 to 35 years after original and consistent exposure. Symptoms may include nasal boils, cysts, perforation of the nose, pharyngitis, sinusitis, and nasal polyps. Since humans do excrete a majority of the Ni to which they are exposed, the concern for the public is not substantial.

The safety level set by the Occupational Safety and Health Administration for $Ni(CO)_4$ gas is 7 $\mu g/m^3$. No safety level has been set for the concentration of Ni in drinking water. Exposure to nickel sulfate and nickel chloride in drinking water caused vomiting and headaches for up to 5 days (Waldron 1980). The metal can cross the human placental barrier, affecting the fetus.

The ionic form of Ni can compete with Ca, cobalt (Co), Cu, Fe, and Zn in compounds, so there is a possibility of interference with such processes as iron absorption. Nickel is inhibitory to a number of enzymes in the human body, including urease and carbon monoxide dehydrogenase. Nickel-sensitive individuals often get contact dermatitis as a result of exposure to jewelry and metal snaps. Workers with increased exposure to Ni include cashiers, ceramic workers, electricians, electroplaters, hairdressers, jewelers, mechanics, and metalworkers or welders. Interestingly, women are reported to be four times more sensitive to Ni dermatitis than men (Costa and Mollenhauer 1980).

Inhalation of Ni compounds has been considered responsible for lung, sinonasal, and laryngeal carcinomas in Ni refinery workers. In the 1930s, researchers found that these workers experienced 16 times the rate of lung cancer and 11 times the rate of nasal sinus cancer compared to the surrounding population (Waldron 1980).

Other illnesses include pneumoconiosis and emphysema. The tracking of refinery workers' health demonstrated exposure to two different forms of Ni: carbonyl gas and Ni processing dust. Nickel may be reabsorbed in the kidney, possibly linking it to some forms of kidney cancer as well. Interestingly, among the different types of Ni compounds, particles (<5 μm) of crystalline nickel subsulfide (NisSa) were carcinogenic, whereas those of the amorphous NiS were not. Particles of the carcinogenic crystalline

Ni_3S_2 were shown to be actively phagocytized by cultures of Syrian hamster embryo cells and Chinese hamster ovary cells, but the cells did take up significant quantities of similar-size particles of the noncarcinogenic amorphous MS (Hong et al. 1997).

Magnesium has been shown to be an effective protector against Ni-induced carcinogenesis in vivo. The mechanisms involved in the protective effect of Mg are unclear. Studies showed that DNA-protein cross-links and chromosomal aberrations in mammalian cells were increased in culture treated with Ni compounds (Yan-Chu 1994). In addition, Ni(II) caused oxidative damage to isolated DNA and chromatin in the presence of H_2O_2, suggesting the formation of reactive oxygen species.

The genotoxic effects of Ni were lessened by added $MgCO_3$. Furthermore, the cells of fibroblasts used in the study showed an 80-fold increase in Ni levels following treatment with nickel subsulfide, but decreased in the presence of $MgCO_3$. These results suggest that the protective role of Mg in Ni-induced cytotoxicity and genotoxicity can be attributed to its ability to reduce either the intercellular Ni concentration or reactive oxygen formation (Yan-Chu 1994).

12.6 ARSENIC

12.6.1 Occurrence and Properties

Arsenic (As) is a ubiquitous element present in various compounds throughout Earth's crust. It is a member of group VA of the periodic table and has the common oxidation states of –3, +3, and +5. The redox states of As are arsenate (H_3AsO_4) and arsenite (H_3AsO_3), which are present in soil solutions. Trivalent As is more soluble and mobile than the pentavalent form (Yan-Chu 1994). Microorganisms can be responsible for the oxidation and reduction of As. These include strains of *Bacillus* and *Pseudomonas* (Hanzlik 1981). *Penicillin brevicaule,* called the arsenic fungi, can produce toxic and highly volatile arsenes. Fungi, yeasts, and bacteria can methylate As into monomethylarsonate, dimethylarsinate, and gaseous derivatives of arsine that are widely distributed in soils (Takamatsu et al. 1982). Methylation of As to organoarsenicals also occurs in invertebrates and vertebrates, including humans. Estimated levels of As in different sources are

- seawater: 2 to 5 ppb
- public water supplies: about 5 ppb (recommended limit is 10 ppb)
- uncontaminated soil: about 5 ppm
- human food: plant sources less than 0.5 ppm; fish/seafood much higher.

An estimated average dietary intake of As in the United States is about 0.9 mg/day, and total body burden of As in an adult is about 15 to 20 mg.

12.6.2 Uses of Arsenic

The use of arsenical compounds increased greatly during the eighteenth and nineteenth centuries. Arsenical compounds were preferred for the control of agricultural pests before the widespread use of organochlorines and organophosphates (Webb

1966). For example, Paris Green, ($[CH_3COO]_2Cu \cdot 3Cu[AsO_2])_2$, and lead arsenate ($PbHAsO_4$), were used as insecticides; white arsenic As_2O_3 was used as a rodenticide; sodium arsenite (a solution of NaOH and As_2O_3) has been used in various pharmaceutical substances. The use of As in veterinary medicine as a nutritional supplement and in the treatment of disease dates to the fifteenth century (Webb 1966). For the past century, the chronic feeding of small doses of various As preparations has been reported to increase appetite, improve the level of activity, correct anemia, and improve the coats of animals. Arsenic was used as a feed additive to aid in the prevention and control of certain enteric diseases of swine and poultry and to improve weight and feed efficiency of livestock in general (Webb 1966). Other miscellaneous uses of arsenic include as pigments and dyes, as preservatives of animal hides, in glass manufacture, and as wood preservatives. Currently, veterinarians employ an organic arsenical, sodium capasolate, for the treatment of heartworms in dogs (Atkins 1992).

12.6.3 Sources of Exposure to Arsenic

Arsenic can be emitted into the environment from several natural sources, including volcanic eruption. Weathering and the processes of sedimentation lead to a wide, natural distribution in water. Pentavalent arsenic (As^{5+}) is slightly more mobile in soil at pH 5.8. As pH rises, trivalent arsenic (As^{3+}) becomes more mobile than As^{5+}, although both increase in mobility. Thought to be pollution free and environmentally friendly, geothermal wells, used as a source of energy, are also a source of arsenic in surface waters. Forest fires can disperse arsenicals to the wind (Smith et al. 1977).

Combustion of fossil fuels and smelting of nonferrous metals are unintentional pathways of removing arsenic, generally as arsenic trioxide, into the environment. Arsenic occurs in most coals in association with sulfur. When burned, arsenic, along with other trace elements, accumulates on fly ash particles. Although found only as trace amounts in coal, the amount present on fly ash is significant (Smith et al. 1977).

Sulfur is often present in mine tailings. Oxidation of sulfur compounds leads to the formation of an acidic solution that can dissolve many elements, including As. The arsenic can then leach into ground- and surface waters (Piver 1983).

12.6.4 Human Exposure to Arsenic

Arsenic is present in urban air at levels of about 0.02 $\mu g/m^3$, and in soil at levels ranging from 0.2 to 40 μg/g. The standard for As in U.S. water supplies as set by the EPA is 50 ng/L. Although most drinking water supplies in the United States contain levels lower than 5 μg/L, about 350,000 people might consume water containing a higher level of As than the EPA standard (Smith et al. 1977).

For the general population, the main exposure to inorganic As is through ingestion. Both organic and inorganic arsenics are present in varying amounts in food. Fish, for example, contain relatively high concentrations of organic arsenic. However, inorganic forms of arsenic can exist as either arsenate or arsenite. Although arsenate is less toxic, it can be converted to arsenite in humans through metabolism.

Arsine (AsH_3) is a colorless, nonirritating gas that is liberated from any arsenic-containing ore that is treated with an acid. Workers in smelters, ore refineries, and areas that are involved with galvanizing, soldering, etching, and Pb plating are at greater risk (Fowler and Weissberg 1974; Vahter 1983).

12.6.5 Animal Exposure to Arsenic

Arsenic enters into animals through three routes: the respiratory tract, the gastrointestinal tract, and the skin. In ambient air, As exists mainly as an inorganic form but is also present in methylated forms at low levels (Tam et al. 1979). The depth of penetration into the lungs depends on the size of As particles and its chemical form. Fly ash particles are small enough to be deposited in the pulmonary region of the respiratory tract. Water-soluble forms of As, such as sodium arsenic and dimethylarsenic acid, are readily absorbed. Large As-laden airborne particles may be removed by the mucocilliary apparatus only to be transported to and absorbed in the gastrointestinal tract (Vahter 1983).

The solubility of As compounds determines the degree of absorption in the gastrointestinal tract. In general, greater than 90% of As^{3+} or As^{5+} given as a water solution is absorbed (Vahter 1983; Yu et al. 1999). In experimental cats and rats, As absorption is highest in the small intestine, whereas oral and gastric absorption is low. Absorption through skin is poorly documented. There are incidents reported that indicated arsenic trichloride and arsenic acid were absorbed. Acute dermal injury by arsenic acid enhances absorption (Tam et al. 1979).

12.6.6 Distribution of Arsenic in the Body

After absorption, inorganic arsenic is transported by blood to other organs. A single oral dose of inorganic arsenic results in elevated As concentrations in liver, kidneys, lungs, and intestinal mucosa (Vahter 1980). Humans who have died of acute arsenic poisoning have had the highest levels found in the liver, kidney, intestinal mucosa, and spleen (Vahter 1983).

The relatively even distribution of As, experimentally administered in different valence forms, is probably due to methylation in the liver and distribution as dimethylarsinic acid. If given at high doses, metabolism is saturated, and inorganic forms are then distributed. Arsenic concentrations are high in skin, hair, and nails. Samples taken from these organs have often been used for exposure analysis. Administration of organic arsenicals to experimental animals results in high levels of arsenic in liver, kidney, and spleen. However, levels are lower in erythrocytes when compared with animals dosed with inorganic forms (Vahter 1983).

Some As, predominantly in the pentavalent form, is excreted directly in feces. Once absorbed, As^{5+} is excreted by the kidneys. Trivalent arsenic is readily excreted in bile to the intestines, where it is available for reabsorption or fecal elimination.

12.6.7 Toxicity of Arsenic

12.6.7.1 Toxicity to Plants

Although research on the toxicity of As to plants is limited, available information indicates that As is potentially toxic to certain plant species. For instance, Fargasova

(1994) studied the acute toxicity of five metals (Cr^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, and As^{5+}) on the germination of mustard seeds (*Sinapis alba*) and found that after 72 hours the most toxic metal for seed germination was As^{5+}. Similar results were obtained from laboratory experiments in which mung bean (*Vigna radiata*) seedlings were exposed to As^{3+}, As^{5+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, and Zn^{2+} for 72 hours, respectively. Arsenic proved to be far more damaging to mung bean germination than other metals tested (Yu et al. 1999).

12.6.7.2 Toxicity of Arsenic to Animals and Humans

The toxicity of As to mammals is related to its absorption and retention in the body and varies with chemical form. The toxicity of arsenicals in decreasing order is as follows: inorganic arsenites > organic trivalent compounds (arsenoxides) > inorganic arsenates > arsonium compounds > elemental arsenic (Eisler et al. 1994). Toxicity appears to be related to the solubility of the arsenical in water. The low toxicity of elemental arsenic is attributed to its near insolubility in water and body fluids. Trivalent arsenic is much more toxic than pentavalent arsenic (Yan-Chu 1994). Arsenate is well absorbed and rapidly eliminated, mainly in urine. Arsenite is also well absorbed but is retained in greater quantities and for longer periods in tissues.

Subsequent to absorption, methylation as well as interconversion between arsenate and arsenite occurs in the body. The interconversion occurs via cytochrome c and cytochrome oxidase (Vahter 1983; Eisler 1994). Specifically, arsenate (As^{+5}) is reduced to As^{+3}, which is then methylated to monomethylarseneic acid and then to dimethylarseneic acid. Methylation occurs mostly in the liver and is facilitated by the presence of S-adenosyl-methionine (SAM). The methylated metabolites are less toxic and less cytotoxic and are more readily excreted in urine.

Arsenite orally administered to mice was found to cross the blood-brain barrier, leading to modified metabolism and function of the CNS, as evidenced by increase in the arsenic trioxide content in discrete brain areas and increased metabolites of norepinephrine and dopamine in the cerebral cortex (but decreased levels in the corpus striatum). Metabolites of 5-hydroxytryptamine increased in all the discrete brain areas. The vertical and horizontal motor activity was increased (Itoh et al. 1990).

Capillary injury and dilation also occur, resulting in transudation of fluid, which in turn decreases blood volume and causes circulatory collapse. Blackfoot, a disease endemic in Taiwan, is caused by As and characterized by the loss of circulation to the extremities, resulting in gangrene (Eisler 1994). Arsenic-induced capillary changes within the kidneys cause tubular degeneration.

Inorganic As compounds are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans (USDHHS 2002). Many cases of skin cancer have been reported among people exposed to As through medical treatment with inorganic As^{3+} compounds. An association between environmental exposure to As through drinking water and skin cancer has been observed and confirmed. Epidemiological studies in areas where drinking water contained 0.35 to 1.14 mg/L arsenic showed increased risks for cancers of the bladder, kidney, skin, liver, lung, and colon in both men and women. Occupational exposure to inorganic As, especially in mining and copper smelting, has consistently been associated with an increased risk of cancer. An almost 10-fold increase in the incidence of lung cancer was observed in workers most heavily exposed to As. Humans exposed to inorganic arsenicals may also have increased risk of cancers in lymph and hematopoietic tissues (Eisler 1994).

Several populations are at risk for high incidences of skin cancer due to water contamination, particularly Chileans and some Taiwanese. Cancer is highest among elderly persons who show symptoms of chronic As poisoning. In general, the incidence of As-induced cancer is dramatically lower in animals (Eisler 1994). However, As is known to be a teratogen in several species of animals, as well as in humans.

A variety of fungi and bacteria has been shown to methylate inorganic As. If the As is in the pentavalent form, it is reduced to arsenite, and methyl groups are added. In animals, As^{5+} appears to be reduced to As^{3+} in the kidney. Methylation then occurs in the liver. Methylation is considered the major detoxification mechanism for inorganic pentavalent arsenates and trivalent arsenites in mammals. Methylated arsenicals rapidly clear from all tissues except the thyroid (Eisler 1994).

Workers at greater risk include those in smelters and those with work associated with pesticides. Chromated copper arsenate (CCA) is the most common form of As used as wood preservative. There were concerns that As, as a known carcinogen, may leach from wood and expose children to unsafe levels. The EPA reached a voluntary agreement with industry to phase out the use of CCA to treat wood for residential structures by the end of 2003.

12.6.8 BIOLOGICAL EFFECTS OF ARSENIC

Cells accumulate As by using an active transport system normally used in phosphate transport (Eisler 1994). Once absorbed, As toxicity is generally attributed to the trivalent form. Toxic effects are exerted by reacting with sulfhydryl enzyme systems (Webb 1966; Eisler 1994). The tissues rich in oxidative metabolism, such as the alimentary tract, liver, kidney, lung, and epidermis, are therefore most affected. Like Pb, Cd, and Hg, discussed previously, As is toxic to living organisms primarily because of its ability to react with and inhibit SH enzyme systems. Figure 12.6 shows the reaction between arsenite (AsO_3^{3-}) and two glutathione (GSH) molecules. The resultant strong covalent bond (–S–As–S–) effectively eliminates the GSH molecules from further reactions.

In animals, As^{5+} appears to be reduced to As^{3+} in the kidney. This is followed by methylation in the liver. Methylation is considered the major detoxification mechanism for inorganic arsenates and arsenites in mammals. Methylated arsenicals rapidly clear from all tissues except the thyroid. Arsenite inactivates enzymes such as

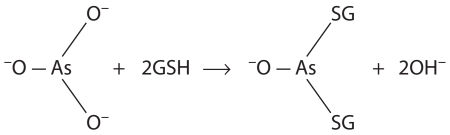

FIGURE 12.6 Interaction of arsenite with two molecules of glutathione.

dihydrolipoyl dehydrogenase and thiolase, resulting in inhibition of pyruvate oxidation and β-oxidation of fatty acids (Eisler 1994; Belton et al. 1985).

Arsenic, as As^{5+}, acts as an uncoupler of oxidative phosphorylation and substrate-level phosphorylation associated with glycolysis. For example, a key step in glycolysis is the conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. An inorganic phosphate (Pi) participates in this reaction. Arsenate (AsO_4^{3-}), which closely resembles Pi in structure and reactivity, can replace phosphate in attacking the energy-rich thioester intermediate, as follows:

$$\text{Glyceraldehyde 3-phosphate} + HAsO_4^{2} \rightarrow \underset{\text{arsenophosphoglycerate}}{AsO_4\text{–GAc–P}} \tag{12.8}$$

The product of Reaction 12.8, l-arseno-3-phosphoglycerate, is unstable and rapidly hydrolyzed. Hence, in the presence of arsenate, the net reaction is

$$\begin{gathered}\text{Glyceraldehyde 3-phosphate} + NAD^+ + H_2O \rightarrow \\ \text{3-phosphoglycerate} + NADH + 2H^+\end{gathered} \tag{12.9}$$

An arsenic-containing poison gas called lewisite was reported to have been used in World War I. Attempts by British scientists to develop a compound to counteract lewisite led to an understanding of how As acts as a poison and subsequently to the development of an antidote. After recognizing that lewisite poisoned people because of the reaction between As and the protein –SH group, the scientists set out to find a suitable compound that contained a highly reactive –SH group that could compete with the protein –SH groups for As, thus rendering the poison ineffective. The research culminated in the discovery of a compound known as British antilewisite (BAL). It is now known that BAL is a chelating agent and, as such, can react with some metal ions and As (Figure 12.7).

There has been a growing interest among biologists and biochemists in the As-induced oxidative stresses as a possible mechanism to explain some of the adverse effects observed with As toxicity. However, the mechanisms involved remain unclear. Zaman and Pardini (1995) reported that both As^{3+} and As^{5+} alter the activities of SOD, catalase, glutathione S-transferase, and glutathione peroxidase (GSHPx). They subsequently have used the activities of these enzymes as markers for As toxicity tests for invertebrates.

Studies by Pi et al. (2002) demonstrated that, in humans, chronic exposure to high levels of As from drinking water elevated serum lipid peroxide (LPO) levels

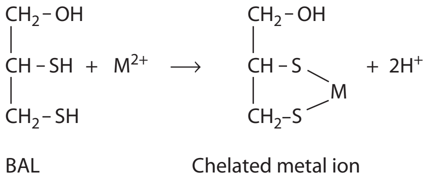

FIGURE 12.7 British antilewisite (BAL) chelation of a heavy metal ion.

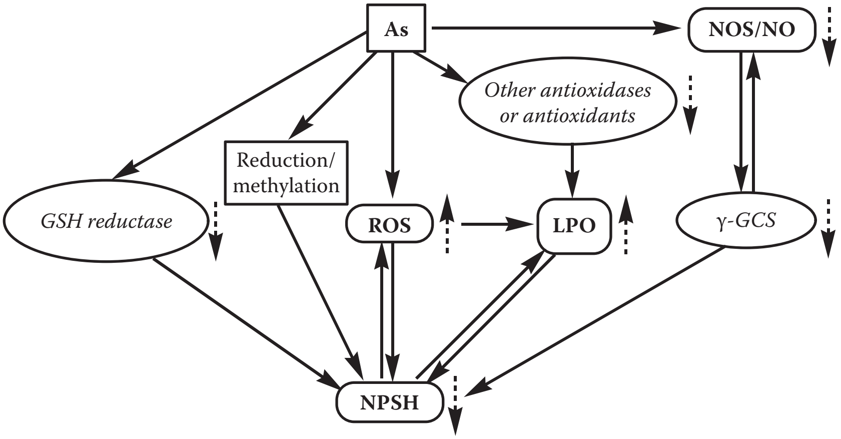

FIGURE 12.8 Arsenic in drinking water and oxidative stress. NOS = nitric oxide synthase; NO = nitric oxide; ROS = reactive oxygen species; γ-GCS = γ glutamylsysteine synthetase. (Adapted from Pi, J., H. Yamauchi, Y. Kumagai, G. Sun, T. Yoshida, H. Aikawa, C. Hopenhayn-Rich, and N. Shimojo. 2002. Evidence for induction of oxidative stress caused by chronic exposure of Chinese residents to arsenic contained in drinking water. *Environ. Health Persp.* 110, 331.)

and lowered nonprotein sulfhydryl (NPSH) levels, suggesting oxidative stress. This observation was derived from epidemiological and biochemical studies on the residents from two villages in Inner Mongolia, China. Residents from the first group had been chronically exposed to 0.42 ppm inorganic As (iAs) in their water supplies (high-As-exposed group), whereas the second group had been consuming 0.02 ppm iAs in their water supplies (low-As-exposed group). The outcome of the study was that residents of the high-As-exposed group had significantly increased LPO concentrations, but decreased NPSH levels compared with those of the low-As-exposed group. As expected, the serum iAs levels of the high-As-exposed group were much higher than those of the low-As-exposed group (Figure 12.8).

REVIEW QUESTIONS

1. Which metals are generally considered the most toxic?
2. Which metal is designated by EPA as one of the six criteria air pollutants?
3. Why are lead shots in lakes and in the field a health risk for birds?
4. What is meant by a *systemic poison*?
5. Why are children more vulnerable to Pb exposure than adults?
6. What is the main reason that Pb is inhibitory to many enzymes?
7. Name the two enzymes involved in heme synthesis that are inhibited by Pb.
8. What is itai-itai-byo? Also, briefly explain how it occurred.
9. What are the main adverse health effects of Cd on humans?
10. What is the basis of Cd toxicity in humans and animals?
11. What is metallothionein? How is it related to Cd exposure in humans?
12. Describe the two mechanisms involved in enzyme inhibition by Cd.

13. What is phytochelatin? How does it act in plants?
14. Explain how dietary protein may be related to the toxicity of ingested Cd in humans.
15. Explain how biomethylation of Hg occurs.
16. Explain the toxicity associated with inhaled Hg vapor.
17. What is the most toxic form of nickel?
18. What types of cancer are considered to be related to Ni inhalation?
19. Which is more toxic, arsenite or arsenate? Can arsenate be converted to arsenite in the body?
20. How do As^{3+} and As^{5+} affect the antioxidant enzymes?
21. What is the importance of methylation of arsenites and arsenates in mammals?
22. Concerning the toxicity of As, how is it related to phosphorus in the tissue?
23. What are lewisite and antilewisite?

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13 Pesticides and Related Materials

13.1 INTRODUCTION

Pesticides refer to any chemicals intended to prevent, deter, destroy, or otherwise impair the ability of pests to compete with desired organisms such as crops, animals, or humans. A *pest*, in turn, is any organism—plant, animal, or microorganism—that is destructive or troublesome or living where it is unwanted. Pesticides can be classified in different ways, but classification based on the target is perhaps the most widely known, as the following examples indicate: insecticides, herbicides, fungicides, and rodenticides (Table 13.1). This chapter considers the chemistry, characteristics, and health effects of several representative groups of pesticides and herbicides. It then discusses several halogenated hydrocarbons that have become of much concern to us in recent years, including polychlorinated biphenyls (PCBs) and dioxins.

13.2 INSECTICIDES

13.2.1 INTRODUCTION

Insecticides are generally defined as those compounds that are effective against insects. Many insecticides have been developed and used to control various species of insects. While most insecticides are applied as sprays, others are applied as dusts, aerosols, fumigants, and baits. The majority of insecticides used today are synthetic organic chemicals, and most of them are nerve poisons. They act by inhibiting the organism's enzymes or interacting with other target sites vital to the proper functioning of the insect's nervous system. Other insecticides act by blocking essential processes such as respiration. Although there are many synthetic organic insecticides, in this chapter three main groups of insecticides are reviewed: hydrocarbons, organophosphorus compounds or organophosphates, and carbamates.

13.2.2 CHLORINATED HYDROCARBONS

13.2.2.1 Introduction

Chlorinated hydrocarbons, also called organochlorines, were the first commercial organic insecticides to be developed. DDT, aldrin, chlordane, dieldrin, endrin, lindane, and heptachlor are some examples (Figure 13.1).

TABLE 13.1
Classification of Pesticides

Method of Classification	Example
By target	Insecticides, herbicides, fungicides, rodenticides, algaecides, nematocides
By chemical nature	Natural organic compounds, inorganic compounds, chlorinated hydrocarbons, organophosphates, carbamates
By physical state	Dust, dissolved solutions, suspended solutions, volatile solids
By mode of actions	Contact poisons, fumigants, stomach poisons

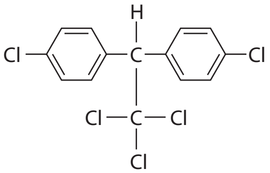
13.2.2.2 DDT

DDT (2,2-bis [p-chlorophenyl]-l,l,l-trichloroethane or dichloro-diphenyl trichloroethane), discovered as a pesticide in 1939, is probably the most widely known pesticide of the twentieth century. It was first used for controlling disease-carrying insects, such as mosquitoes that spread malaria. As the range of DDT's effectiveness against insects became known, it was used by soldiers during World War II to control the spread of typhus by body lice. After World War II, DDT was used in the home and applied to a variety of agricultural crops, providing enormous success in pest control. DDT proved effective in the control of a large number of pests, including the gypsy moth, potato pests, the corn earthworm, and codling moths. Because of DDT's impact on human disease control, the discoverer of DDT, Dr. Paul Muller, received the Nobel Prize in Medicine in 1948. Despite these successes, some 20 years later when DDT's environmental consequences became evident, its use was either limited or totally banned in industrialized countries, although it is still used in a number of less-developed countries.

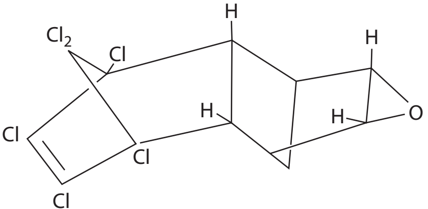
DDT is characterized by its very low vapor pressure, extremely low solubility in water (1.2 ppb), and high solubility in oils. Because of this last property, DDT can be readily absorbed through the skin in the fatty tissues of living organisms and can biomagnify as passed through the food chain. DDT is released slowly, when the stored fat is called on as a source of energy. Of the two isomers of DDT, the *p,p′*-isomer is more toxic to invertebrates than the *o,p*-isomer.

DDT and other chlorinated hydrocarbons are typically persistent broad-spectrum insecticides. Their residues persist in the environment for long periods of time, ranging from a few months to years. The half-life ($T_{1/2}$) of DDT, for instance, is estimated to be 7 to 30 years, depending on the environment. The organochlorines have broad-spectrum characteristics, enabling them to affect many different species of insects. Environmental persistence of this group of chemicals is because they are not readily degraded by the action of water, heat, sunlight, or microorganisms. DDT rapidly accumulates in invertebrates to several thousand times the exposure level in extremely low concentrations. The 96-hour LC_{50} (concentration that kills 50%) for 19 species of fish ranges from 1.8 to 22 μg/L (Table 13.2). A 60% reproductive impairment was observed in *Daphnia* at 100 μg/L.

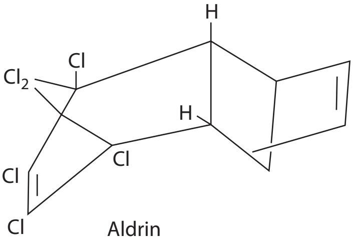
DDT adversely affects several physiological characteristics, including normal ratios of serum ammo acids, thyroid activity, and the ability to withstand stress.



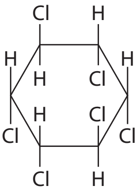
p, p´-DDT (Dichloro-diphenyl trichloroethane)



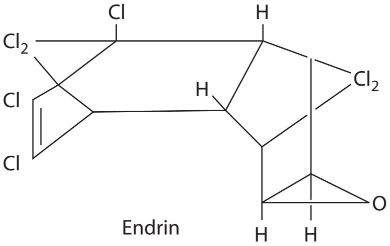
Dieldrin



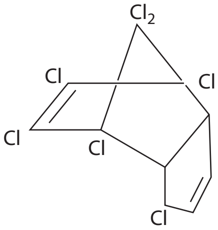
Aldrin



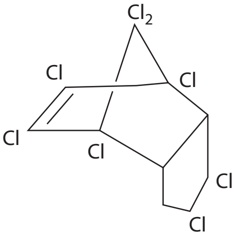
Lindane (1, 2, 3, 4, 5, 6-hexachlorocychlohexane)



Endrin



Heptachlor



Chlordane

FIGURE 13.1 Chemical structures of chlorinated hydrocarbon insecticides.

TABLE 13.2
Summary of Acute Toxicity of DDT for Fish

Test Organism	Stage or Weight (g)	96-hour LC_{50} (HE/L)
Black bullhead	1.2	4.8
Bluegill	1.5	8.6
Channel catfish	1.5	21.5
Coho salmon	1.0	4.0
Fathead minnow	1.2	2.2
Largemouth bass	0.8	1.5
Northern pike	0.7	2.7
Rainbow trout	1.0	8.7
Walleye	1.4	2.9
Yellow perch	1.4	9.0

Although DDT was not shown to influence gonad maturation, the mortality of fry produced by DDT-treated parents was high, especially during the terminal stages of yolk absorption (Johnson and Finley 1980).

DDT and other chlorinated hydrocarbons are very resistant to metabolic breakdown. Nevertheless, in animals and humans, DDT is degraded to DDE (ethylene 1,1-dichloro-2,2-bis(p-chlorophenyl) or dichlorodiphenyl dichloroethylene) or DDD (ethane l,l-dichloro-2,2-bis(p-chlorophenyl)) (Figure 13.2). A limited conversion of DDT to DDE occurs in human subjects. The conversion is catalyzed by DDT dehydrogenase, and the resultant DDE is a stable metabolite.

Research conducted by Redetszke and Applegate (1993) further demonstrated the persistence and biomagnification of chlorinated hydrocarbons. These researchers studied the residues of organochlorine pesticide in adipose tissue samples of 25 persons (19 males and 6 females) from El Paso, Texas. None of the tissue was taken from people known to have occupational exposure to pesticides. Eight organochlorine compounds were observed in the tissue samples. The pesticide residue levels were in the moderate range. DDE was found in all the samples tested, with an average level of 4.96 ppm, whereas the average level of DDT was 1.50 ppm. Since DDE is a stable breakdown product of DDT (Figure 13.2), its presence in the tissue represents mainly past ingestion. It could also represent low-level indirect exposure from food and water from areas where DDT was used in the past and persists in the environment.

Nakata et al. (2002) studied the levels of persistent organochlorines such as DDTs, hexachlorocyclohexanes (HCHs), chlordane compounds (HCLs), and hexachlorobenzene (HCB) in a wide variety of foodstuffs and human tissues collected from Shanghai and its vicinity in China between 2000 and 2001. Among the organochlorine compounds analyzed, DDT and its metabolites were found to be prominent in most of the foodstuffs. In particular, mussels were found to contain 34 ppb (based on lipid weight) of DDTs, levels that were one to three orders of magnitude greater than those reported in bivalves from other Asian countries. The levels of the other compounds in foodstuffs were found to be generally low, suggesting relatively small

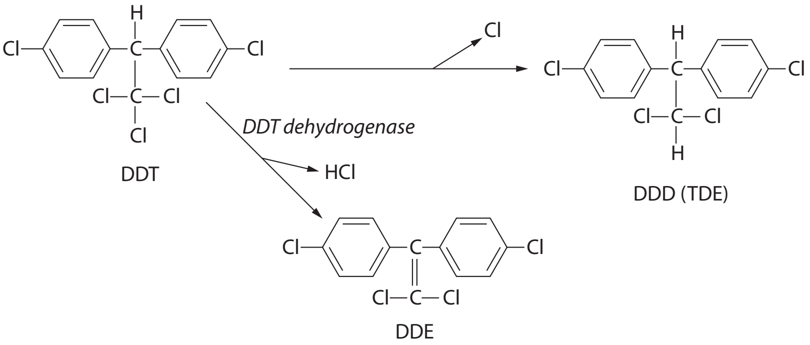


FIGURE 13.2 Metabolism of DDT.

amounts of inputs into the environment. However, the researchers found high concentrations of DDTs and HCHs in human tissues from Shanghai, with the maximum values of 19 ppb and 17 ppb (lipid weigh), respectively. The researchers concluded that, because foodstuffs are a main source of human exposure to contaminants, the greater concentration of DDTs and HCHs in the Chinese residents under study might be due to extensive use of these compounds as agricultural pesticides in the past (Nakata et al. 2002).

One of the most important health effects of DDT, DDE, and a number of other chlorinated hydrocarbons is on the endocrine system. Many studies have provided evidence suggesting that chlorinated hydrocarbon residues found in the environment may be responsible for interference with the functioning of the endocrine system and disruption of reproduction. Published reports related to such disruption involve alligators in Lake Apopka, Florida; seagulls in Tacoma and bald eagles on the Columbia River (both in the state of Washington); and trout in the United Kingdom, among others. Louis Guillette, a zoologist, was credited with the initial observation that many of the Lake Apopka alligators exhibited abnormal reproductive systems and meager male hormones, apparently due to pesticide residues (Hileman 1999). Field and laboratory studies have shown similar effects of a number of toxicants on wildlife. Observed effects include

- feminization of male alligators and trout when exposed to hormone-like chemicals in laboratories
- poor reproduction among bald eagles along the Columbia River (seemingly linked to exposure to DDE and PCBs; see Section 13.4)
- offspring of exposed pregnant females showing elevated testicular cancer and delayed puberty (in mice), malformed sex organs (in rats), and reduced sperm counts (in hamsters)
- salmon in the Great Lakes with enlarged thyroids and males with premature sexual development

Some scientists suggested that exposure to these chemicals could be related to the surge of disorders in human reproductive organs—from declining sperm counts to increasing breast and prostate cancers—in the industrialized world since World War II. (Chapter 15 deals with more detailed information on endocrine disrupters.)

The adverse effects of organochlorine compounds on birds have been widely known ever since Rachel Carson published *Silent Spring* (Johnson and Finley 1980). Not all species of birds have suffered equally, however. Birds of prey are especially susceptible to the persistent organochlorine insecticides, and the levels that inhibit reproduction can be very much lower than those that kill. For example, common species used in the laboratory, such as chicken, pheasant, pigeon, or sparrow, can cope with insecticides far more successfully than other species. Birds that migrate lay down large amounts of fat prior to migration to serve as a store of energy for the long journey. Because many pesticides are soluble in fat, birds accumulate the poison in their fat before migrating, and the poison is released to do its damage when fat is consumed during the journey.

Delegates from about 110 countries met in Geneva in September 1999 to work on a treaty to control 12 persistent organic pollutants (POPs). They agreed to the international phaseout of the pesticides aldrin, endrin, and toxaphene. They also decided to severely restrict the use of four others—chlordane, dieldrin, heptachlor, and mirex—and one industrial chemical, hexachlorobenzene, allowing only some residual uses. Countries are aiming for a global treaty because these persistent bioaccumulative chemicals can be transported by wind and water far from where they are originally used and can cause damage to wildlife. Even at low doses, these chemicals also are suspected of causing diseases of the immune system, reproductive disorders, and abnormal child development in humans. However, the countries were unable to make a decision on DDT, PCBs, dioxins, and furans. The World Health Organization (WHO), public health specialists, and some developing countries wanted DDT kept available for malaria control until equally inexpensive alternatives are developed (Hileman 1999).

The U.S. Environmental Protection Agency (USEPA) has revealed new limits on three pesticides commonly used on Western farms to protect endangered and threatened Pacific salmon. The restrictions apply to the use of chlorpyrifos, diazinon, and malathion near salmon waters in Washington, California, Oregon, and Idaho. (Chlorpyrifos is known by trade names such as Dursban and Lorsban. Malathion is sold under trade names such as Fyfranon and Celthion. Diazol has one trade name under which it is sold: Diazinon.) The chemicals have been found by the U.S. Geological Survey to interfere with the salmon's sense of smell, making it harder for them to find food, avoid predators, and return to native waters to spawn, according to federal biologists.

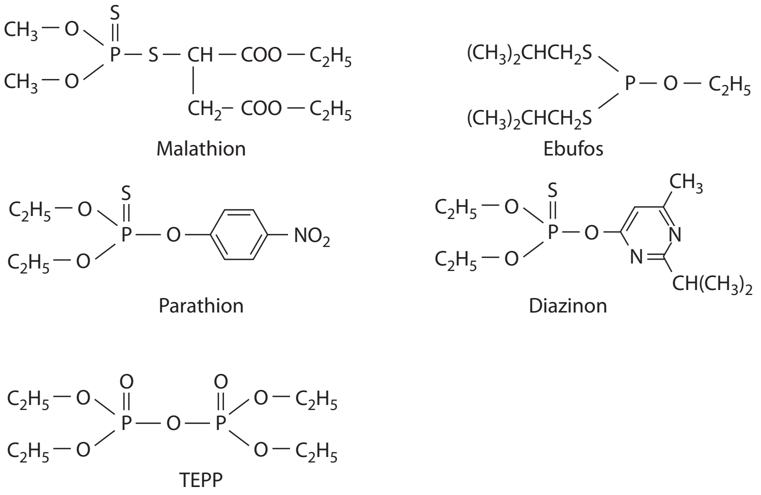
The new rule prohibits the use of these pesticides within a range of 100 ft (30 m) and 1,000 ft (300 m) of salmon waters, depending on size of the river or stream, application rate, and other criteria. The new regulations come after antipesticide groups and salmon fishers sued the federal government in 2001 for not considering the impact of pesticides on federally protected salmon and steelhead.

According to the National Oceanic and Atmospheric Administration (NOAA) Fisheries Service, the three chemicals are banned from household use, but tens of millions of pounds are still applied on a wide range of fruit, vegetables, cotton, and livestock to control termites, mosquitoes, flies, and other pests.

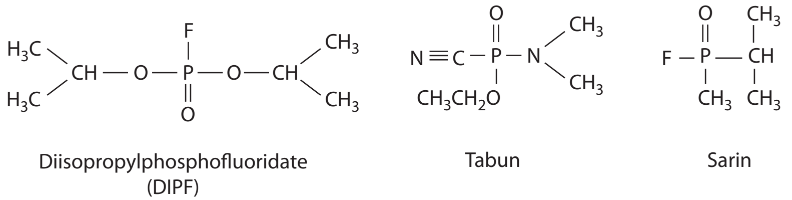
13.2.3 ORGANOPHOSPHORUS COMPOUNDS

13.2.3.1 Introduction

Organophosphorus insecticides are the most toxic among the insecticides; they are dangerous not only to insects but also to mammals. Many of these compounds, such as parathion, paraoxon, timet, and tetram, are in the "supertoxic" category of human poisons. Human fatal doses for these toxicants are less than 5 mg/kg, along with arsenic (As), cyanide (CN^-), and some others. As little as 2 mg of parathion has been known to kill children. Figure 13.3a shows the chemical structure of three representative organophosphorus insecticides: parathion, malathion, and tetraethyl pyrophosphate (TEPP). Figure 13.3b shows several organophosphorus compounds or organophosphates: diisoproylphosphofluoridate (DIPF), sarin, and tabun. These are highly toxic but are not used as pesticides. Sarin and tabun are nerve gases used in chemical warfare. Diisopropylphosphofluoridate was initially intended for use in chemical warfare but was excluded because of its relatively lower toxicity compared with the other two agents.



(a) Insecticides



(b) Nerve gases

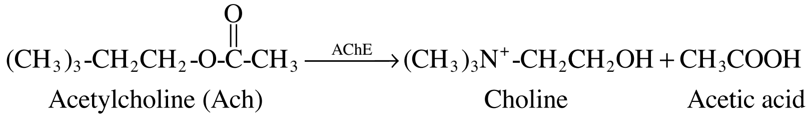
FIGURE 13.3 Chemical structures of organophosphate insecticides (a) and nerve gases (b).

13.2.3.2 Toxicity of Organophosphorus Compounds

Organophosphorus insecticides are very toxic, and exposure-related health problems have been encountered, especially in the earlier days of application. Symptoms of poisoning in humans include nausea, vomiting, diarrhea, cramps, sweating, salivation, blurred vision, and muscular tremors. Severe cases may be fatal due to respiratory failure. Even though organophosphates are usually more toxic to humans and mammals than chlorinated hydrocarbons, they are more easily biodegraded than the organochlorines. Because they do not persist in the environment or accumulate in fatty tissue, they have virtually replaced the organochlorines for most uses (American Chemical Society [ACS] 1987).

13.2.3.3 Action of Acetylcholinesterase and Organophosphates

The mode of action of organophosphorus insecticides in vertebrates and invertebrates is the inhibition of *acetylcholinesterase* (AChE), the enzyme responsible for breaking down the neurotransmitter acetylcholine (ACh). ACh, in turn, is produced from choline and acetyl coenzyme A (CoA) by choline acetyltransferase (Equations 13.1 and 13.2). Inhibition of the enzyme results in accumulation of ACh at the nerve endings, leading to disruption of nervous activity. As shown in the reactions, subsequent to breakdown by AChE, ACh is regenerated from choline. The resultant acetic acid is activated to acetyl CoA before reacting with choline.



$$\underset{\text{Acetylcholine (Ach)}}{(CH_3)_3\text{-}CH_2CH_2\text{-}O\text{-}\overset{\overset{\displaystyle O}{\|}}{C}\text{-}CH_3} \xrightarrow{\text{AChE}} \underset{\text{Choline}}{(CH_3)_3N^+\text{-}CH_2CH_2OH} + \underset{\text{Acetic acid}}{CH_3COOH} \quad (13.1)$$

$$\text{Choline} + \text{Acetyl CoA} \xrightarrow{\text{Cholineacetyl transferase}} \text{Acetylcholine} \quad (13.2)$$

Because of the important role that AChE plays, it is worthwhile reviewing the principles of nerve transmission. The junctions between adjacent neurons are termed *synapses* (Figure 13.4). Nerve impulses, also called *action potentials*, are transient changes in the membrane potential that move rapidly along nerve cells. Action potentials are created when the membrane is locally depolarized by about 20 mV. This small change is sufficient to dramatically influence the specific proteins in the axon membrane called *voltage-gated ion channels*. These proteins are ion channels that are specific either for sodium ions (Na^+) or potassium ions (K^+). The ion channels are normally closed at the resting potential of −60 mV. When the potential difference rises to −40 mV, the "gates" of the Na^+ channels will be opened, causing Na^+ ions to flow into the cell. The membrane potential continues to increase after the entrance of Na^+ ions, opening additional Na^+ channels. In this way, the action potential moves down the axon in a wavelike manner. The potential rises to more than +30 mV, then the influx slows and stops. As the Na^+ channels close, K^+ channels begin to open, and K^+ ions rush out of the cell, returning the membrane potential to negative values. The potential eventually overshoots its resting value, when K^+ channels close. The

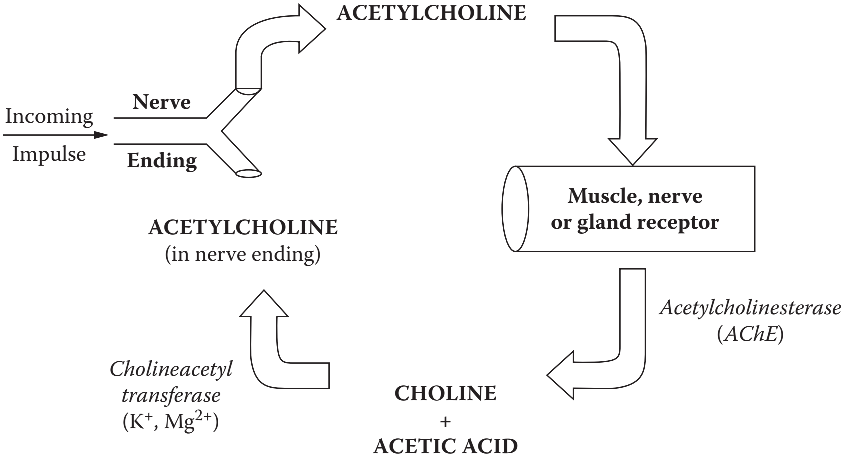

ACETYLCHOLINE
Incoming
Impulse
Nerve
Ending
Muscle, nerve or gland receptor
ACETYLCHOLINE (in nerve ending)
Acetylcholinesterase (AChE)
Cholineacetyl transferase (K^+, Mg^{2+})
CHOLINE + ACETIC ACID

FIGURE 13.4 Diagrammatic representation of the action of acetylcholine and acetylcholinesterase.

resting potential is eventually restored by the action of the Na^+,K^+-ATPase (adenosine triphosphatase) and the other channels (Garrett and Grisham 1995).

The cell-to-cell communication at the synapse is mediated by ACh. A brief summary of this system of communication follows:

1. The arrival of an action potential at the synaptic knob opens Ca^{2+} channels in the presynaptic membrane.
2. Influx of Ca^{2+} induces the fusion of ACh-containing vesicles with the plasma membrane and release of ACh into the synaptic cleft.
3. Binding of ACh to receptors in the postsynaptic membrane opens Na^+ channels.
4. The influx of Na^+ depolarizes the postsynaptic membrane, generating a new action potential.

AChE has a reactive serine at the active site that is a vulnerable target for organophosphate inhibitors. Inhibition of the enzyme results in accumulation of ACh at the nerve endings, causing disruption of synaptic activity. Evidence indicates that the vertebrate AChE contains two binding sites, and it is likely that the insect enzyme is similar. The anionic site, which may contain a glutamate residue, interacts with the positively charged nitrogen (N) atom of ACh, while the esteratic site is responsible for the cleavage of the ester link of ACh. The esteratic site contains a serine residue, whose nucleophilicity is enhanced by hydrogen bonding to the imidazole group of a neighboring histidine residue. Chemicals such as organophosphate insecticides that can inactivate AChE are known to attach to the $-CH_2OH$ residue of the esteratic site of the enzyme by forming a covalent bond. They are therefore often called covalent inhibitors of AChE.

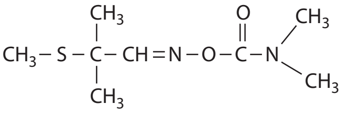


FIGURE 13.5 Chemical structure of aldecarb.

13.2.4 CARBAMATES

In the same way that organophosphate insecticides, such as parathion and malathion, are derivatives of phosphoric acid, the carbamates are derivatives of carbamic acid (HO–CO–NH_2). Carbamates are widely used for worm control on vegetables. Examples of carbamates include aldicarb [2-methyl-2-(methylthio) propionaldehyde-*O*-(methylcarbamoyl) oxime)] and carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate). The mode of action of the carbamates is the same as that of organophosphates, that is, inhibition of AChE.

Aldicarb (trade name Temik) (Figure 13.5) is one of the most widely used carbamates. The first time it was detected in groundwater was in Suffolk County, New York, in August 1979. Although laboratory and field studies indicated that the pesticide could not reach groundwater, a combination of circumstances led the residues to reach groundwater and to be ingested by humans. A monitoring program revealed that 1,121 (13.5%) of the 8,404 wells tested exceeded the state's recommended guideline of 7 ppb. Of the contaminated wells, 52% contained 8 to 30 ppb aldicarb, 32% contained 31 to 75 ppb, and 16% had more than 75 ppb. Studies did not, however, reveal any cases of carbamate poisoning (Zake et al. 1982).

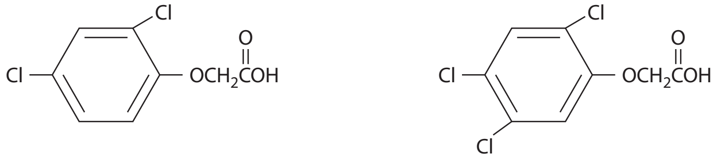
Case Study 13.1

Another aldicarb episode occurred in four western states (California, Washington, Oregon, and Alaska) and one Canadian province (British Columbia) in 1986. About 300 people were made ill over the long July 4 weekend after eating watermelons contaminated with aldicarb. The melons were grown on farms in Southern California. Forty of 550 California State watermelon fields were shown to be contaminated with the pesticide. As a result, about 1 million melons were destroyed. Aldicarb is manufactured by Union Carbide. Its approved use is on a number of crops to control nematodes, aphids, and other insects that feed on parts of crop plants. It is not approved for use on watermelons. It was reported that a concentration of aldicarb at 0.2 ppm in watermelon meat caused illness. The contaminated melons had concentrations up to 3 ppm. Symptoms resembled those of influenza, that is, blurred vision, perspiration, nausea, dizziness, and shaking. These symptoms usually disappear after a few hours. In the episode mentioned, none of the cases proved fatal.

13.3 HERBICIDE

13.3.1 2,4-D AND 2,4,5-T

During the Vietnam War years, the U.S. Air Force defoliation program applied a huge amount of undiluted 2,4-D (2,4-dichlorophenoxy acetic acid) and 2,4,5-T



2,4-D (2,4-dichlorophenoxy)acetic acid
(a)

2,4,5-T (2,4,5-trichlorophenoxy)acetic acid
(b)

FIGURE 13.6 Chemical structures of (a) 2,4-D and (b) 2,4,5-T.

(2,4,5-trichlorophenoxy acetic acid) (Figure 13.6) on Vietnam's crop and forestland between 1965 and 1970. In addition to military use of the phenoxyherbicides (PHs) in Vietnam, PHs were widely used in the United States for weed control in agriculture and rangeland, in lakes and ponds, and in forestry.

As shown in Figure 13.6, 2,4-D and 2,4,5-T are identical esters except for the additional chlorine atom present on the benzene ring of 2,4,5-T. During production of these two compounds, chlorinated dioxins (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) (discussed in Section 13.6) were found to contaminate the final product, a compounding factor in analysis because of its high toxicity. Prior to its ban in 1978, 2,4,5-T was used in combination with other chemicals in forestry primarily for "releasing" conifer species from competition with broadleaved species. PHs are also used after logging to clear the brush so that seedlings can be planted.

In plants, the biochemical actions of PHs are complex. After application, the chemicals are absorbed primarily through stomata and secondarily through root hairs with water. In resistant species, detoxification results after various decarboxylation and conjugation steps. In sensitive plants, as the chemicals are translocated through vascular tissue, they disrupt growth and various metabolic processes. The most important change is the stimulation or inhibition of many enzymes, which in turn affects growth and metabolic processes, possibly leading to plant death. Certain species, such as Douglas fir, are tolerant when PHs are mixed with a water carrier.

Numerous clinical reports in humans have described peripheral neuropathy (degeneration of nervous tissue) and acute myopathy (disorder of muscle tissue or muscles) after dermal exposure or oral ingestion of 2,4-D. Clinical symptoms of severely poisoned farmers include pain and weakness in the lower extremities, slowed nerve conduction velocity, twitching, and muscle spasms. In addition, behavioral changes such as nervousness, inability to concentrate, irritability, impotence, and others may occur (Goldstein et al. 1969). These symptoms have also been found in other studies involving workers employed at PH manufacturing plants. In the early studies, the degree of TCDD contamination was often unknown. In later studies, exposure is primarily due to the formulated product.

The neurotoxic and mycotoxic mechanisms of 2,4-D are not well studied (Berwick 1970). In recent years, several investigations have been made involving *nerve conduction velocity* (NCV) measurement. This approach has become increasingly valuable in xenobiotic assessment because slowed NCV is associated with histological as well as behavioral changes. NCV is an excellent starting point for epidemiology because the techniques involved are rapid, accurate, and noninvasive. In 1979, a survey was conducted of 190 current, former, and retired workers of a Jacksonville, Arkansas, plant where PHs had been produced for 20 years (Singer et al. 1981). Workers and control subjects were carefully screened to minimize factors that could possibly affect NCV. Three nerves were tested (median motor, median sensory, and sural), measured, and recorded for 56 workers at the plant. The results showed that 46% of the study group had one or more slowed NCVs. In addition, a slowed sural NCV was correlated to duration of employment at the factory.

The widespread use of PHs during the Vietnam War has been associated with a large variety of health problems. Again, TCDD is a complexing factor. Specific neurotoxic effects of 2,4-D have been examined in response to reports of episodic increase in intracranial skull pressure associated with insecticide intoxication (Sanborn et al. 1979). These symptoms prompted the first research involving central neural metabolism of 2,4-D, specifically concerning the accumulation and transport within the brain and spinal cord.

PHs were banned for forestry in 1979 due to a combination of public pressure and the results of the USEPA's Alsea II report (Wagner et al. 1979). This widely criticized report found significantly greater spontaneous abortion rates inside a residential area exposed to PH spray compared to a similar area without spray. Although banned for use in forestry, PHs are still widely used as herbicides for cotton, corn, wheat, and rice crops.

13.3.2 ATRAZINE

Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine (Figure 13.7), is a widely used herbicide. However, its use is controversial due to its effects on nontarget species, such as on amphibians, and because of widespread contamination of waterways and drinking water supplies. Although it has been excluded from a reregistration process in the European Union, it is still one of the most widely used herbicides in the United States and the world.

Atrazine is used to stop pre- and postemergence broadleaf and grassy weeds in major crops. The compound is both effective and inexpensive and thus is well suited to production systems with very narrow profit margins, as is often the case with maize. Atrazine functions by binding to the plastoquinone-binding protein in photosystem II, which animals lack. Plant death results from starvation and oxidative damage caused by breakdown in the electron transport process. Atrazine degrades in soil primarily by the action of microbes. The half-life of atrazine in soil ranges from 13 to 261 days. The oral median lethal dose or LD_{50} for atrazine is 3,090 mg/kg in rats, 1,780 mg/kg in mice, and 1,000 mg/kg in hamsters.

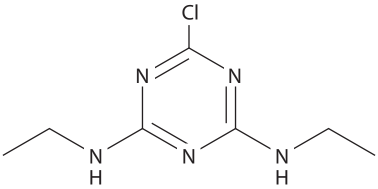


FIGURE 13.7 Chemical structure of atrazine.

Atrazine was banned in the European Union in 2004 because of its persistent groundwater contamination. In the United States, however, it is one of the most widely used herbicides, with 76 million pounds of it applied each year, in spite of the restriction that used to be imposed. It is probably the most commonly used herbicide in the world and is used in about 80 countries. Its alleged endocrine disruptor effects, possible carcinogenic effect, and epidemiological connection to low sperm levels in men has led several researchers to call for banning it in the United States (USEPA 2003). The EPA reviewed the safety of the herbicide in 2006 and declared it to be safe for use on corn and other crops when used as directed. But, in fall 2009 the agency decided to reevaluate the potential health effect of atrazine because of studies that suggested an association between exposure to the pesticide and birth defects; premature births, and low birth weight in humans. Widespread use of the herbicide on crops such as corn has made it the most commonly detected pesticide in U.S. streams and groundwater also.

Over the course of 2010, EPA's Office of Pesticide Programs (OPP) attempted to integrate everything there is to know about the safety of atrazine, including animal toxicity data and, for the first time, human epidemiological data, looking at both cancer and noncancer effects. The agency will decide whether new restrictions on the chemical are necessary (Erikson 2010).

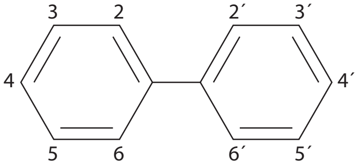
13.4 POLYCHLORINATED BIPHENYLS

13.4.1 Introduction

Polychlorinated biphenyls (PCBs) are a class of synthetic chlorinated organic compounds with biphenyl as the basic structural unit. Chlorination of the basic structure can theoretically yield 209 chlorobiphenyls substituted with 1 to 10 chlorine atoms, but the probable number of compounds is estimated to be 102. The general chemical structure of PCBs is shown in Figure 13.8. Although PCBs belong to chlorinated hydrocarbons, they are not pesticides. However, because of their wide use and resistance to degradation in the environment, PCBs are known as one of the major organochlorine pollutants found in the environment. Extensive PCB contamination exists in the food chain throughout the world.

13.4.2 Properties of PCBs

The properties of PCBs are similar to those of DDT. PCBs are soluble in fat or fat solvents but are hardly soluble in water. The solubility of PCBs in water and in



PCB

FIGURE 13.8 Chemical structures of PCBs (numbers are possible sites for Cl).

organic solvents affects their transport and persistence in the environment. Their solubility in water generally decreases with increase in the degree of chlorination. Individual chlorobiphenyls vary in their solubility from about 6 ppm for monochlorinated biphenyls to as low as 0.07 ppm for octachlorobiphenyls (Waid 1986). They are nondrying and nonflammable (they are stable on long heating at 150°C), do not support combustion when alone above 360°C, and can withstand temperatures up to 650°C (1,600°F). They are not affected by boiling with NaOH solutions. Electrically, PCBs are nonconducting. PCBs also have very low vapor pressures, which, like their solubility in water, decrease with increased chlorination.

PCBs tend to bind tightly to particulate matter, such as soils and sediments. Therefore, surface waters with low particulate loads may have very low concentrations of PCBs, while high concentrations may exist in bottom sediments.

13.4.3 Uses of PCBs

PCBs were first manufactured commercially in 1929 in the United States by the Monsanto Chemical Company using the trade name Aroclor followed by serial numbers (such as 1221, 1248, and 1268, etc.). The last two digits in the serial numbers refer to the percentage of chlorine in the products. This nomenclature has since been replaced by the International Union of Pure and Applied Chemistry (IUPAC) PCB nomenclature. Appendix 2 presents a summary of the nomenclature for this group of compounds.

Because of their unique properties, PCBs were widely used. Industrial uses include manufacture of plastics, paints, varnishes, asphalt, rubber, carbon paper, carbonless paper, printing inks, synthetic adhesives, sealers in waterproof material, lubricating oils, fire retardants, electrical transformers, and capacitors in the power industry (D'Itri and Kamrin 1983). Although PCBs are not pesticides, they were previously added to DDT to extend its "kill effect."

The United States banned the use of PCBs in 1976 in the wake of concern about public health. In 1985, the EPA issued a final rule requiring removal of PCB fluids, or electrical transformers containing PCBs, from commercial buildings by October 1, 1990.

13.4.4 Environmental Contamination of PCBs

Like DDT, PCBs are ubiquitous in the environment. Contamination of PCBs may occur through various activities, including

- spills and losses in manufacture of PCBs and PCB-containing fluids
- vaporization or leaching from PCB formulations
- leaks from sealed transformers and heat exchangers
- leaks of PCB-containing fluids from hydraulic systems that are only partially sealed
- disposal of waste PCBs or PCB-containing fluids (Nisbet and Sarofim 1972)

In addition, PCBs are released into the air or waterways by the incineration of rubber and plastics and through the use of pesticides that contain added PCBs.

One of the most important routes by which PCBs can contaminate the environment is *air.* Airborne PCBs can rapidly and efficiently dissipate from point sources to distant areas. In addition to the airborne route, marine environments receive PCBs from various sources, including rivers, urban runoff, wastewater discharges, and dumped sewage sludge. Like DDT, once in the aquatic environment, PCBs tend to bioaccumulate. PCBs and DDT are similar to each other in terms of their low water solubilities, extreme lipophilicity, and great resistance to degradation (Waid 1986a).

Gambaro et al. (2005) studied the concentrations of PCBs over an austral summer at a site in Terra Nova Bay, Antarctica. The scientists found that gas-phase concentrations of PCB congeners in the atmosphere of Terra Nova Bay ranged from below the detection limit to 0.25 pg m^{-3}, with a mean concentration of *total* PCB of 1.05 pg m^{-3}. The PCB profile was dominated by lower chlorinated PCB congeners; in fact, more than 78% of the total PCB content was due to congeners with one to four chlorine atoms and only about 10% with five to seven chlorine, whereas higher chlorinated PCB congeners were below detection limits. These observations led the researchers to hypothesize that PCB local source contributions were not very important, whereas long-distance transport was the prevalent factor bringing PCBs to Terra Nova Bay (Veith and Lee 1971).

13.4.4.1 Wildlife Exposure to PCBs

PCBs were identified in birds' feathers as early as 1944, and many investigators have since reported varying levels in wildlife in Canada, Germany, Great Britain, Japan, the Netherlands, Sweden, and the United States. High concentrations of the compounds have been found in fish taken from the Great Lakes (Stow et al. 1994), Hudson River, and Tokyo Bay. Polar bears and fish in the Arctic tundra lakes also contain PCB residues, as do birds living in Antarctic waters.

The presence of PCBs in the Great Lakes is still of considerable concern, even though the use and manufacture of PCBs were banned in the 1970s. Concerning the risk to the Great Lakes system, a new index based on fate, persistence, and toxicity ranked PCBs second to dioxins. The primary concern for the public is the danger of PCBs present in consumable fish. Studies carried out by the Wisconsin Department of Natural Resources on coho and chinook salmon in Lake Michigan showed general decreases in the PCB levels between 1974 and 1990. For example, the highest sample mean for coho PCBs was found in fish samples obtained in 1976, with a value of 14.25 mg/kg, while the highest sample mean for chinook PCBs occurred in 1974, with a value of 11.69 mg/kg. Sample means in 1990 decreased to 0.83 and 1.17 mg/kg for coho and chinook, respectively (Otto and Moon 1996).

However, PCB concentrations in fish are related to a number of factors, such as the size, fat content of the fish, and the food web structure. Furthermore, slower-growing fish can accumulate higher levels of contaminants than faster-growing fish. This is because faster-growing fish gain more body mass for each unit mass of contaminant they consume than do slower-growing fish.

The decreases in PCB concentrations mentioned appear to be diminishing, and there is concern that a slow increase in PCB concentrations in the fish is now occurring. Although the reasons for this change are not well known, some researchers suggested that the increase may be related to the decline in the alewife population in Lake Michigan that began in the early 1980s. Since alewife is an important food source for both coho and chinook salmon, it is suspected that the decline in alewife has led to slowed growth in coho and chinook, leading to increased levels of PCBs (Otto and Moon 1996).

Otto and Moon (1996) collected brown bullheads *(Ameiurus nebulosus)* from the St. Lawrence River and compared their detoxification capacities to bullheads from a relatively nonpolluted aquatic system, Lac La Peche in Canada. They observed that the content of PCBs in white muscle was significantly higher (22-fold) in bullheads from the St. Lawrence River compared with those from Lac La Peche. Activities of liver *ethoxyresorufin O-deethylase* (EROD) were 2.8-fold higher in St. Lawrence River bullheads than in fish from Lac La Peche. (As noted previously, EROD is widely used as a biomarker for pollution by synthetic organic compounds, particularly chlorinated hydrocarbons.)

13.4.4.2 Human Exposure to PCBs

Human exposure to PCBs is the combined result of intake from air, water, and food sources, the majority being attributable to consumption of fish (except for sporadic instances of contamination). Exposure through inhalation is not likely to exceed 1 mg/day, and the amount taken in drinking water is at most 5 to 10 mg/day (Nelson 1972). Thus, even in highly industrialized areas these represent minor sources of PCB intake. According to EPA market basket surveys during the 1970s, the average adult in the United States received 5 to 10 mg PCB/day in the diet (Kolbys 1972). The value fluctuates widely because PCBs are found primarily in meat, poultry, and especially fish products. Individuals who eat large quantities of fish or who eat fish from areas polluted two or three decades ago would have intakes in excess of 100 mg/day.

The most highly documented case of PCB poisoning in humans is known as *yu-sho* or “oil disease,” which occurred in southwest Japan in 1968. The disease was caused by ingestion of rice oil contaminated by a commercial brand of Japanese PCB, Kanechlor 400. This particular PCB brand contained 48% chlorine and was found in the contaminated rice oil at concentrations from 2,000 to 3,000 ppm (Masuda 1985). By the end of 1982, more than 1,700 persons were poisoned.

Another highly documented case of PCB poisoning was that called *yu-cheng* (the Chinese for “oil disease”), which occurred in central Taiwan in 1979. Again, contaminated cooking oil was the source. By the beginning of 1983, there were 2,060 persons identified as victims. The total average intake of PCBs by the victims of yu-sho and yu-cheng was estimated to be 633 mg and 973 mg, respectively (Masuda 1985).

High levels of PCBs were found in seafood collected from the Columbia River during 2009, according to the Washington State Health Department. Officials advised against eating fish, including perch, flounder, English sole, and crab. The EPA found PCB levels well above safety thresholds, an average 716 ppb in the skinless fillet of an English sole, for example. Tests between 1992 and 1999 found an average of 267 ppb.

13.4.5 METABOLISM OF PCBs

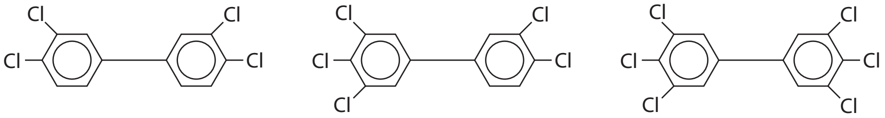
In humans and animals, PCBs are absorbed from the gastrointestinal tract and distributed rapidly to all tissues. Elimination of the absorbed PCBs from the body occurs slowly, with its extent dependent on the number of chlorine atoms on the PCB molecule. Like other polycyclic aromatic hydrocarbons, PCBs are metabolized by the microsomal mixed-function oxidase (MFO) system. Through hydroxylation and conjugation with gluculonic acid, the polarity of the PCB molecules is enhanced, thereby increasing their solubility in body fluids and allowing excretion (Safe 1984). This process is strongly dependent on the location and degree of chlorination of the biphenyl molecule. The rate of metabolism and excretion decreases as the number of chlorines increases. Therefore, monochlorobiphenyls are metabolized and excreted faster than dichlorobiphenyls, which are processed faster than tetrachlorobiphenyls. The degree of chlorination also affects how PCBs are eliminated from the body: mono- and dichlorophenyls are largely excreted in the urine, whereas PCBs with higher numbers of chlorine atoms are excreted primarily in the feces (Safe 1984).

When the number of chlorine atoms on the biphenyl molecule is four or more, the position of the chlorine atoms becomes important in determining the rate of metabolism and excretion of the PCB species. The primary requirement for more rapid metabolism is the presence of two adjacent unsubstituted carbon atoms on the biphenyl molecule.

Like DDT and its metabolites, PCBs stored in adipose tissue are mobilized into the liver under starvation stress. Because PCBs are metabolized in the liver, the health of the liver is critical. When the liver cells are damaged by certain drugs or toxicants, such as CCl_4, the liver will not be able to perform its detoxification process effectively. A possible route of environmental breakdown of PCBs is through photolysis or a photochemical process. PCBs absorb ultraviolet (UV) radiation in the 200- to 300-nm range, leading to declorination. This causes PCBs to be converted to a less-harmful state, the biphenyl product. Several factors influence the photolysis, notably the degree of chlorination, position of Cl substitution in the ring, and environmental factors. Although photolysis of certain PCB analogs has been demonstrated experimentally, the extent to which the reaction occurs in the environment is less known. Environmental degradation of PCBs also occurs in soils, lakes, rivers, and sediments by the activities of both aerobic and anaerobic microorganisms. As a result, less-chlorinated chlorobiphenyls are produced. The main mechanism involved in the biodegradation is hydroxylation, while ring cleavage may also occur.

13.4.6 TOXICITY OF PCBs

Studies indicated that the toxicity of technical PCB mixtures may be due to the presence of trace levels of several PCB congeners with four or more Cl atoms at *both para* and *meta* positions in the biphenyl rings but no Cl atoms in *ortho* positions (Safe 1984). Among the 20 possible coplanar PCB congeners 3,3′,4,4′-tetrachlorobiphenyl, 3,3′,4,4′,5-pentachlorobiphenyl, and 3,3′,4,4′, 5,5′-hexachlorobipheynyl (Figure 13.9) were found to be the most toxic. These three coplanar congeners and dioxin were considered responsible for eliciting toxic effects in experimental animals, including



3,3',4,4'-Tetrachlorobiphenyl

3,3',4,4',5-Pentachlorobiphenyl

3,3',4,4',5,5'-Hexachlorobiphenyl

FIGURE 13.9 Chemical structures of three coplanar PCB congeners.

body weight loss, dermal disorder, hepatic damage, thymic atrophy, teratogenicity, reproductive toxicity, and immunotoxicity (Urabe and Asoki 1985).

The symptoms reported in both yu-sho and yu-cheng episodes included increased whitish eye discharge and swelling of the upper eyelids; pigmentation of nails, skin, and mucous membranes; acne-like skin eruption (chloracne) with secondary infections; feelings of weakness; headache; and vomiting. Three to four years after both incidents, the skin of those people who were only mildly poisoned appeared normal, yet systematic disorders, including dullness, cough, headache, stomachache, and swelling and pain of the joints, persisted (Hirayama 1975). By 1984, of the people poisoned in Taiwan 24 had died of liver cirrhosis or hepatomas. In addition, 39 babies born to women who had been poisoned suffered from hyperpigmentation, and 8 of them died soon after birth. Those children who did survive showed obvious signs of growth retardation. Of the yu-sho victims, 112 people had died by the end of 1982. However, the causes of only 31 deaths were confirmed; 11 were from neoplasms, primarily of the stomach, liver, and lung (Masuda 1985). Other clinical manifestations of PCB poisoning include dental, endocrine, neurological, and hematological disorders. PCBs can also cause developmental defects such as learning problems in children and harm the immune system.

13.4.7 BIOLOGICAL EFFECTS OF PCBs

Studies have shown that PCB poisoning led to metabolic changes in human victims. These changes may be caused primarily by the dysfunction of metabolic organs and secondarily by accelerated metabolism through enzyme induction. For example, exposure to PCBs causes an altered general lipid metabolism. An elevated concentration of serum triglyceride was commonly observed among victims of PCB poisoning. Since a significant positive correlation occurred between the triglyceride concentration and the blood PCB concentration, it is suggested that PCBs may be responsible for the hypertriglyceridemia. The hypertriglyceridemia appears to be due to disturbance of plasma triglyceride removal caused by diminished lipoprotein lipase following PCB exposure (ACS 1983).

PCBs, like other chlorinated hydrocarbons, exhibit high binding affinity to hepatic cytosolic receptor protein *(Ah* receptor) and induction potency of hepatic microsomal enzymes (Urabe and Asoki 1985). An increase in hepatic microsomal enzymes may result in an increased metabolism of endogenous substances, including some hormones. For instance, PCBs have been reported to cause an increased degradation of estradiol, as evidenced by the lowered serum levels of the hormone among the Japanese female victims of PCB poisoning.

PCBs cause heme depletion by inhibiting uroporphyrinogen decarboxylase, an enzyme involved in heme synthesis (see Chapter 12). Because such depletion has a negative-feedback effect, it increases the synthesis of ALA synthetase, which ultimately leads to uroporphyrin accumulation in the liver. PCBs also influence the metabolism of vitamin A. In animal experiments, rats fed diets containing 20 ppm PCBs showed decreased storage of vitamin A. Suggested mechanisms for the decline include PCB-induced reduction of serum retinol-binding protein and increases in microsomal enzymes that metabolize vitamin A.

13.5 POLYBROMINATED BIPHENYLS

13.5.1 Introduction

Polybrominated biphenyls (PBBs) are another group of halogenated aromatic hydrocarbons and were used predominantly as flame retardants in thermoplastics. About 5,000 tons of the material were manufactured from 1970 to 1975 in the United States.

Between May and June 1973, a chemical company in Michigan mistakenly sent 227 to 454 kg (500 to 1,000 pounds) of PBBs to a grain elevator in south Michigan in place of magnesium oxide, a livestock feed additive. Subsequently, the PBBs were mixed into feed for cattle and other farm animals, which were then slaughtered and sent to market, ultimately contaminating a majority of the state's population. The contamination necessitated slaughter of more than 35,000 head of cattle, 1.6 million chickens, and thousands of pigs on 1,000 Michigan farms. The total damage cost was $500 million. Since Michigan is a meat-, milk-, and egg-deficit state, the contamination was, for the most part, limited to Michigan. Because of this event, PBBs are no longer manufactured in the United States.

13.5.2 Chemistry of PBBs

There are numerous PBB isomers, but commercial products usually have one to six bromine atoms. The chemical structures of several representative PBB isomers are shown in Figure 13.10. PBBs are lipophilic, poorly metabolized, and slowly excreted. The metabolites are hydroxyl derivatives. When a dose of monobromobiphenyl was injected into rabbits, 1% of the compound was found as a hydroxylated metabolite.

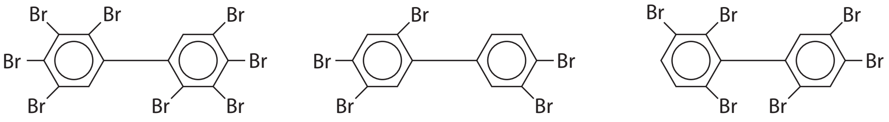


FIGURE 13.10 Chemical structures of several PBB isomers.

13.5.3 Toxicity of PBBs

PBBs are extremely persistent. When ingested, they remain in the body fat, perhaps indefinitely. They are toxic to the skin, kidneys, testicles, and adrenal gland. They cause liver damage, including liver tumors, and birth defects. In cows, milk production is decreased, coats become rough, and hoof deformities occur.

In humans, the ailments vary between individuals. Observable symptoms include nervousness, sleepiness, weakness, fatigue, lethargy, severe headaches, memory loss, nausea, joint swelling, and pain in the back and legs. Disorders in the skin, such as dryness, and nail discoloration occur. Gastrointestinal problems are common. In a survey of 2,000 individuals selected to be representatives of the population of Michigan, more than 90% had PBB concentrations of higher than 10 ppb in body fat, while members of the general population had no detectable levels. The FDA declared less than 0.3 ppm as the safety level in meat and dairy products, and if the concentration is beyond that level, animals were to be quarantined by the state.

13.5.4 Biological Effects of PBBs

Like PCBs, PBBs are potent inducers of hepatic microsomal drug-metabolizing enzymes. In the cell, PBBs act on mitochondria and disrupt energy production of all cellular processes. Clinical observations among the contaminated farmers in Michigan showed an elevated activity of serum glutamate-oxaloacetate transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), and lactic acid dehydrogenase (LDH). Immunological studies showed decreases in absolute number and percentage of T and B lymphocytes and significant reduction of in vitro immune function. Interestingly, however, neither the subjective nor the objective findings correlated with either serum or fat PBB levels.

13.6 DIOXIN

13.6.1 Introduction

Dioxin refers to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and is a congener of the family of PCDDs. PCDDs and PCDFs, unlike PCBs, have not been purposely manufactured. Rather, they are present as impurities associated with the synthesis of chlorophenols. PCDDs are one of the most toxic substances known and, like PCBs, are ubiquitous in the environment. There are 75 dibenzo-7-dioxins containing chlorine atoms. Figure 13.11 shows the general structure of PCDDs.

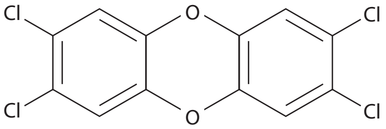


FIGURE 13.11 Chemical structure of 2,3,7,8-tetrachlorodibenzo-*p*-doxin (TCDD).

13.6.2 Exposure to PCDDs

Human exposure to PCDDs has been associated with workers engaged in the manufacture of technical chlorophenols and their derivatives, such as the herbicide 2,4,5-T (Wenning et al. 1993). The main sources of PCDDs in the environment include combustion-related processes, municipal waste and medical waste incinerators, pentachlorophenol formulations, numerous industrial manufacturing and chemical formulation processes, fires, and urban runoff and stormwater (Wenning et al. 1993). The formation of PCDDs by pyrolysis of PCBs and chlorinated benzenes was observed in 1982 as the result of an electrical transformer fire in Binghamton, New York.

Humans are exposed to dioxin through herbicides in the air and soil; consumption of fish and meats; improper industrial waste disposal such as occurred in Times Beach, Missouri; and industrial accidents such as the chemical plant accident in Seveso, Italy. However, the most well-known human exposure to the chemical is that of the defoliant Agent Orange, used in the Vietnam War. Agent Orange, a combination of the herbicides 2,4-D and 2,4,5-T, was sprayed over the dense jungles of Vietnam to clear brush and trees that provided cover to the enemy. The herbicide was contaminated with small amounts (average 2 ppm) of TCDD.

13.6.3 Toxicity of Dioxins

13.6.3.1 Toxicity of Dioxins in Animals

The acute toxicity of TCDD, exhibited by LD_{50} in a number of laboratory animals, varies considerably with species. For example, the LD_{50} for guinea pigs is 0.6 mg/kg body weight, whereas the values for the mouse and hamster are 114 and 5,000 mg/kg body weight, respectively. Perhaps one of the unique characteristics of dioxin is that it has different effects on different species. Major symptoms exhibited in animals include

- abnormal cell proliferations or organ enlargement, such as seen in lung, skin, gastric mucosa, intestinal mucosa, urinary tract, and bile duct/gallbladder
- atrophy or decreased cell proliferation in thymus, bone marrow, and testicle
- other effects such as liver lesions and edema (ACS 1983).

In rodents, adverse effects on reproduction, immune function, lipid and glucose metabolism, and behavior have also been reported (Schmidt 1992). Guinea pigs exposed to dioxins exhibit loss of lymphoid tissue, particularly from thymus, thus becoming more susceptible to infections, although death does not result from infections. They die from a starvation-like wasting of the entire animal. Liver damage is less severe. Dioxins can inhibit sex hormones and may also induce adverse effects on insulin, increasing the chance of diabetes (Gibbons 1993). Chronic effects of dioxins in animals also vary with species. For example, PCDDs may be fetotoxic to some (e.g., monkeys), but teratogenic to others, such as mice. However, the high toxicity of dioxins to the mother means that the range in which dioxins cause toxic effects on the fetus but not on the mother is very narrow. Thus, some toxicologists classify dioxins as a weak teratogen (Zake et al. 1982). Ironically, the fact that humans appear to be less sensitive to the acute effects of dioxins means that it could be a more potent teratogen for them than it seems to be for laboratory animals.

The toxicity of dioxins varies widely from species to species, but all exposed animals studied commonly exhibited wasting away of tissue. As mentioned, tissue wastage is probably the cause of death in the very sensitive guinea pig. In addition, dioxin exposure may impair cell membrane proliferation.

Earlier studies with animals suggested a strong connection between dioxin and endometriosis (the presence of uterine lining in other pelvic organs, especially the ovaries, characterized by cyst formation, adhesions, and menstrual pains). Scientists at the University of Wisconsin and others demonstrated that monkeys exposed to dioxins developed the disease and that the incidence of the disease correlated with dioxin doses. For example, 71% of monkeys exposed to 25 ppt (parts per trillion) had moderate-to-severe disease, while only 4,294 of animals fed 5 ppt had the disease. By contrast, the control group of animals not fed dioxin had neither moderate nor severe disease (Wiesmuller et al. 2002).

Studies on rats and mice showed that dioxins are extremely potent carcinogens in these animals. Female rats fed varying doses of dioxins were shown to develop liver tumors. In addition, at high doses both male and female rats developed increased numbers of tumors in the mouth, nose, and lungs, as well as in the liver. It is suspected that dioxins may be about three times as potent a carcinogen as aflatoxin B1, which is one of the most potent carcinogens known. In another study, scientists observed increases in thyroid tumors in male rats. Researchers consider that TCDDs may act as a promoter rather than initiator (see Chapter 16).

13.6.3.2 Toxicity of Dioxins in Birds

There was a series of dramatic avian population declines in a number of countries during the late 1940s and early 1950s. The declines were mainly associated with reproductive failure, characterized by marked eggshell thinning, poor hatchability, and lowered numbers of chicks surviving a couple of weeks. Most of these reproductive effects were correlated with exposure to xenobiotics, particularly DDT and dioxin-like compounds. Furthermore, the observed deformity or anatomical malformations were associated with egg concentrations of PCDDs, PCDFs, and dioxin-like PCBs.

Many studies have since been conducted on the contaminations of birds by PCDDs and PCDFs. Wiesmuller et al. (2002) measured the concentrations of PCDDs, PCDFs, and PCBs in unsuccessfully hatched eggs of three species of predatory birds—hobbies, goshawks, and sparrow hawks—collected in the Berlin-Brandenburg region of Germany. By use of TEQs for birds, the researchers found that eggs of hobbies contained mean concentrations of 475 pg TEQ/g fat and 551 pg TEQ/g fat contributed by PCDD/Fs and coplanar PCBs, respectively. The researchers also found that, with the exception of one location, the burdens of TEQ originating from PCDD/PCDFs decreased steadily from 1991 until 1998.

A similar study was conducted on the concentrations of PCDDs, PCDFs, and non- and mono-*ortho*-chlorine-substituted biphenyls (dioxin-like PCBs) in livers of 17 species of birds collected from Japan (Senthilkumar et al. 2002). The birds were grouped into granivores, piscivores, omnivores, and predators based on their feeding habits. The researchers found the ranges of liver concentrations of PCDD/PCDFs by omnivores, piscivores, and predators to be 2,300–8,000 pg/g, 61 to 12,000 pg/g, and 480 to 490,000 pg/g on a fat weight basis, respectively. Livers of granivores contained relatively low concentrations of PCDD/PCDFs (80 to 660 pg/g). According to the authors, this was the first study on those toxicants in livers of several species of birds in Japan (Senthilkumar et al. 2002).

13.6.3.3 Toxicity of Dioxins in Humans

The first studies of dioxins in people were conducted on chemical workers exposed to dioxins, revealing relatively mild acute effects. The observed responses include chloracne and, at high levels of exposure, a general sense of fatigue or malaise, disturbances in the responses of the peripheral nervous system, and liver toxicity, including changes in many enzyme levels and, in some cases, enlargement of the liver. These conditions generally subsided after a few years (Nakata et al. 2002). Although more than 800 workers have been exposed to dioxin in industrial accidents since 1949, no clear case of human death has been shown to be caused by dioxin exposure. However, more recent studies have revealed that dioxin disturbs various aspects of sexuality; has subtle endocrine, developmental, neurological, and immunological effects; and is a potent carcinogen (Gibbons 1993). The mentioned studies of monkeys exhibiting connection between endometriosis and dioxin (Wiesmuller et al. 2002) led to research into the connection in the more than 5 million women in the United States with the disease. The results obtained from the studies have convinced many researchers that what is observed in animal studies also applies to humans.

Researchers in both Milan, Italy, and at the Centers for Disease Control and Prevention in Atlanta, Georgia, reported that exposure to high levels of PCDDs in both parents was linked to an excess of female offspring. As mentioned, an industrial accident in July 1976 released kilogram amounts of PCDDs near Seveso, Italy. Researchers found that, in the zone where the population was most heavily exposed to TCDD, 26 male babies and 48 female babies were born in the period from 9 months after the accident until December 1984. Ordinarily, about 106 males are born for every 100 females. The ratio of males to females returned to normal between 1985 and 1994. The half-life of PCDDs in adults is about 8 years, so it can be assumed that about half of the dioxins were cleared from exposed adults by 1985. No males at all were born to parents who both had measured PCDD blood levels of 100 ppt or higher.

Agent Orange became the center of the health controversy after the Vietnam War. During the 1970s, Vietnam veterans with a variety of illnesses began to blame their medical problems on Agent Orange exposure. According to the report published recently by the Institute of Medicine (IOM), there is “suggestive but limited evidence” that exposure to Agent Orange during the Vietnam War is associated with an increased chance of ischemic heart disease and Parkinson’s disease in veterans. Direct studies linking these health problems to veterans are lacking, however. The IOM strongly recommends that such studies of Vietnam veterans be performed. The report is part of the series of biannual congressionally mandated reviews of Agent Orange exposure and veterans’ health (Whitelock 1993).

Health risks from exposure to dioxin are thought to include cancer, immune system effects, increased likelihood of diabetes, and others. The EPA is in the process of drawing a reassessment of the health risks from exposure to dioxin and related compounds. Here, dioxin refers to 2,3,7,8-tetraclorodibenzo-*p*-dioxin, the most toxic of the dioxin congeners. Related compounds include other dioxins, furans, and PCBs that behave like dioxin in humans and animals.

Dioxin has a long history. As mentioned, dioxin and related compounds gained prominence as contaminants in the herbicide Agent Orange used during the Vietnam War. Dioxin was also discovered to be an inadvertent by-product of many industrial processes, such as pulp and paper manufacture and waste incineration, and it builds up in the environment because it has a 7-year half-life. However, dioxin emissions have declined 92% since 1987, and concentrations in human tissue have fallen 80% since the 1980s. The primary sources of exposure today are beef, pork, fish, and dairy products.

13.6.4 GENE REGULATION BY DIOXINS

The similarity of biological effects of several classes of polychlorinated hydrocarbons, including PCDDs, led to the hypothesis that these compounds may act through a specific receptor (Schmidt 1992). Experiments with mice showed that dioxin induces the cytochrome P450 system and its associated enzymes. Researchers subsequently found that this response is governed by a single autosomal gene with a gene locus that codes for the *Ah* receptor protein. The receptor protein preferentially binds to arylhydrocarbons (Schmidt 1992). A similar receptor has been discovered in human cells (Whitelock 1993). The presence of the *Ah* receptor makes an organism more sensitive to several effects that dioxin and other PCDDs elicit, such as enzyme induction, carcinogenesis, and immunotoxicity. Different *Ah* receptor levels in different animals and genetic strains may explain why dioxin evokes biological responses at different dose levels (Schmidt 1992). These discoveries support receptor-medicated specificity of response.

Current understanding of a probable mechanism of gene regulation by dioxins may be summarized as follows:

1. TCDD first enters the cell through passive diffusion and then binds to the *Ah* receptor, forming a receptor complex TCDD-*Ah*.
2. The TCDD-*Ah* undergoes an unknown transformation or activation step and can subsequently be translocated into the nucleus.
3. In the nucleus, the complex binds to specific regions of core DNA called dioxin-responsive elements (DREs).
4. Binding of the complex to DREs results in increased gene transcription of several genes.
5. The transcribed messenger RNA (mRNA) is then translated in the cytosol, leading to the synthesis of cytochrome P450 enzymes.

This is considered the primary biological response (Figure 13.12). Secondary biological responses include perturbation of hormone systems and altered patterns of cell growth and differentiation (Vanden Heuval and Lucier 1993). Studies have shown a high correlation between laboratory animals and human responses. As mentioned,

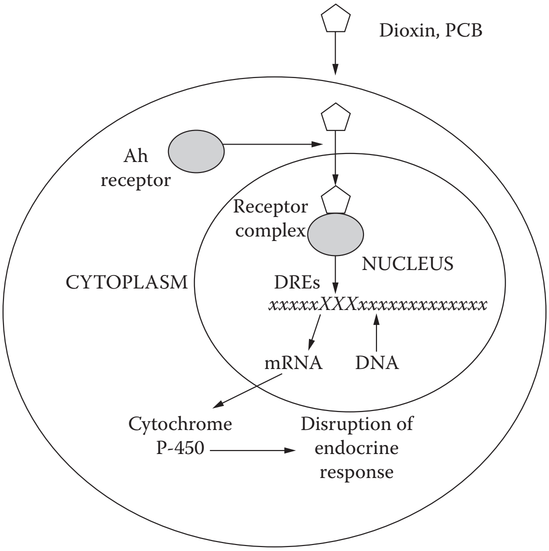


FIGURE 13.12 Proposed mechanism by which dioxins and PCBs effect endocrine disruption.

dioxin is now considered a carcinogen, although it does not damage DNA as most carcinogens do. However, by attaching to the *Ah* receptor and entering the nucleus, dioxin switches on genes that control cell growth and proliferation. Dioxin is also a cancer promoter because it can trigger DNA damaged by other carcinogens to start producing abnormal cells. Therefore, dioxin is considered a potent carcinogen because it can cause a wide variety of cancers, rather than a specific type (Gibbons 1993).

13.6.5 Environmental Degradation of TCDD

Although pure TCDD is extremely persistent, it is not stable as a contaminant in thin herbicide films exposed to outdoor light. Research showed that herbicide formulations containing known amounts of TCDD and exposed to natural sunlight on leaves, soil, or glass plates lose most or all of the TCDD within a single day (Crosby and Wong 1977). It is agreed that three factors are required for dioxin to break down: dissolution in a light-transmitting film, the presence of an organic hydrogen donor (such as a certain solvent or pesticide), and UV light.

REVIEW QUESTIONS

1. What is the mode of action of most insecticides?
2. What are the characteristics of DDT?
3. Compare the characteristics of organophosphates and organochlorines.
4. Why is DDT persistent in the environment?
5. Which is more toxic to humans, organophosphates or organochlorines?
6. What is the mode of action of organophosphates in insects?

7. What are the reasons for organophosphates to be more widely used than organochlorines?
8. Describe the action of acetylcholinesterase (AChE).
9. Which of the following is (are) AChE inhibitor(s)? (a) DDT, (b) parathion, (c) carbamate
10. What is meant by *yu-sho* or "oil disease"?
11. Female victims of PCB poisoning exhibit lowered serum estradiol levels. Explain why.
12. What properties do PCBs share with DDT?
13. What are the environmental sources of TCDD?
14. List the major symptoms exhibited by animals exposed to TCDD.
15. What types of cancer does TCDD elicit?
16. Briefly explain the current understanding of the mechanism by which dioxin is involved in gene regulation.

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14 Occupational Toxicology

14.1 INTRODUCTION

14.1.1 Antiquity to Middle Ages: Diseases among Miners

There are many situations in which workers are exposed to various chemical compounds in the work environment. Over the long history of humans, the effects of toxic compounds related to work have been studied. Hippocrates (460–377 BC) pointed out the occupation of the patient as one of the factors that should be considered in the description of the therapy and overdosages. Lead was used 6,000 years ago, and lead colic, paralysis, visual disturbance, and encephalopathy among lead workers were described in the verse by a Greek poet in the second century BC (Landrigan 1990). Mercury was used 3,000 years ago in China (Clarkson and Magos 2006). In the first century (Roman times), mercury was mined and described as a poison to all living things (Gloag 1981). In the Middle Ages, the health problems miners had were reported. The German scientist Agricola (1566) reported respiratory failure among miners, and in Italy many women had to change husbands several times because their husbands died at young ages working in a mine in the Carpathian Mountains. In 1567, Paracelsus described the etiology of miners' disease in *On the Miners' Sickness and Other Diseases of Miners*. Ramazzini published *Discourse on the Diseases of Workers* in 1700 and summarized occupational diseases among various workers, including miners in metal mines, doctors who used mercury as a drug, and portrait painters who used lead (Ramazzini 1983). The mining diseases occurred all over the world in addition to Europe. Similarly, Sugae, a Japanese folklorist, described that the miners working in the gold mine in Akita, Japan, in the beginning of the nineteenth century, had short lives, with few of them reaching the age of 42, and many of the women married seven or eight times because their husbands died at an early age (Jambor 1999).

14.1.2 After the Industrial Revolution: Metal Diseases

After the Industrial Revolution, which started around 1760 in England, workers were exposed to more varieties of toxic compounds. Parcival Pot in 1775 reported scrotal cancer among chimney sweepers at relatively young ages in London and stated the relationship between the scrotal cancer and soot. This is the first report of chemical carcinogenicity of polyaromatic hydrocarbons contained in the soot. From the eighteenth to the nineteenth century, various heavy metals, such as cadmium, chromium, nickel, and manganese, were discovered and used as industrial materials. Subsequently, the health problems caused by these metals were also reported. In the twentieth century, the production of these metals increased dramatically, and many workers were exposed to them. Without protective apparatus, many of these workers suffered from occupational diseases.

The most representative toxic metal used from the nineteen century is cadmium. The production of cadmium materials began in 1829. In 1858, occupational cadmium poisoning was reported in Russia. After World War I, cadmium electroplating was introduced. Since then, workers' deaths due to chemical pneumonia by the inhalation of cadmium fumes were reported. In addition, because of the frequent use of batteries (nickel–cadmium batteries), cadmium became a more important metal. Friberg (1984) reported chronic cadmium intoxication among battery workers. Since then, the study of chronic toxicity of cadmium has developed. The symptoms of chronic cadmium intoxication include a golden yellow ring in teeth, pulmonary emphysema, and a tubular dysfunction in the kidney with proteinuria. The predominant protein in the proteinuria is β_2-microglobulin. A number of cases with osteomalacia following occupational exposure to cadmium have also been reported. The findings in chronic toxicity of cadmium among workers helped the elucidation of the itai-itai-byo disease, which occurred among inhabitants in Toyama Prefecture in Japan as a result of consuming cadmium-contaminated rice (Gloag 1981; Friberg 1984). The onset of itai-itai disease, a multifactorial disease in which cadmium plays a major role, is manifested by a combination of severe kidney damage and osteomalacia.

For ancient metals, their uses have changed over the course of history, and various occupational health hazards occurred. Lead is a representative ancient metal and is also widely used in modern times. It is used in water pipes, batteries, alloys, glasses, and so on. Industrial lead poisoning was common from the eighteenth to the nineteenth century and was mainly due to inhalation of lead dust or fumes (Landrigan 1990). After the twentieth century, because of the improvement in the work environment and protective apparatus, the number of cases of industrial lead poisoning decreased. However, it is still found even in developed countries. In the developing countries, lead poisoning was observed among people engaged in the recovery of waste metals containing lead. The symptoms of lead poisoning include anemia, gastrointestinal symptoms such as lead colic and constipation, and wrist drop due to radial nerve palsy (Table 14.1). The mechanisms of anemia induced by lead involve decreases in the activities of enzymes associated with heme synthesis, as illustrated in Figure 14.1. The most sensitive index of the toxicity of lead is the decrease in the activity of δ-aminolevulinic acid dehydratase (ALA-D) in erythrocytes (Hemberg

TABLE 14.1
The Dose-Effect Relationship in Lead Intoxication

Lead Concentration in Blood (μg/dL)	Effect
10–20	Decrease in δ-aminolevulinic acid dehydratase (ALA-D) activity in erythrocytes
40	Increase in δ-aminolevulinic acid in urine
80–100	Anemia
80–150	Lead colic, wrist drop due to radial nerve palsy

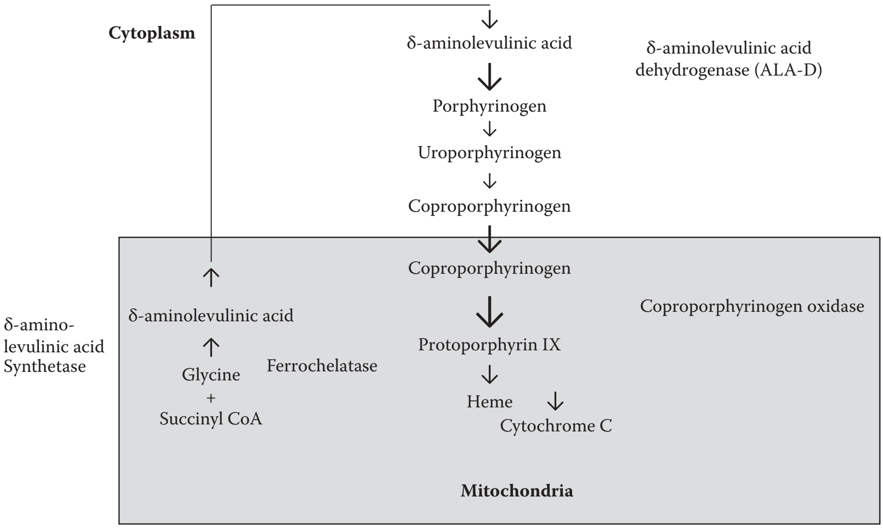

Cytoplasm
δ-aminolevulinic acid
δ-aminolevulinic acid dehydrogenase (ALA-D)
Porphyrinogen
Uroporphyrinogen
Coproporphyrinogen
Coproporphyrinogen
Coproporphyrinogen oxidase
δ-aminolevulinic acid
δ-amino-levulinic acid Synthetase
Protoporphyrin IX
Glycine
+
Succinyl CoA
Ferrochelatase
Heme
Cytochrome C
Mitochondria

FIGURE 14.1 Heme biosynthesis.

et al. 1970; Landrigan 1990). Ferrochelatase is another enzyme in heme synthesis inhibited by lead (Tephly et al. 1978; Landrigan 1990).

14.1.3 AFTER THE NINETEENTH CENTURY: ORGANIC COMPOUNDS, ORGANIC METALS, AND GASES

The era after the nineteenth century is one in which various organic compounds were also synthesized. Since then, many health hazards caused by organic compounds have been reported. By the 1880s, more than 10,000 organic compounds, including tetrachlorocarbon, which induces hepatotoxicity such as hepatic cirrhosis (Pond 1982), became commercially available. After the World War II era, when organic chemistry progressed rapidly, a wide variety of organic compounds was synthesized, and the occupational hazards of these problems became of public health concern for the workers.

In addition, the synthesis of organic metals became popular, and health problems caused by organic metals were reported. For example, in addition to the occupational health hazards by inorganic mercury that have been known from antiquity (Clarkson and Magos 2006), Hunter–Russell syndrome, an occupational methylmercury poisoning, was reported in England in 1940 (Hunter and Russell 1954) and in Sweden in 1948 and 1954. The symptoms of Hunter–Russell syndrome are neurological ones, such as paresthesia (tingling sensation) in the fingertips and around the mouth and lips, constricted visual fields, cerebellar ataxia, speech disturbance, and hearing loss (Clarkson and Magos 2006). In severe cases, these progress to coma and death. In the 1950s, Minamata disease occurred in Minamata, Japan, where many inhabitants who ate fish containing high levels of methylmercury were poisoned. The common symptoms between Hunter–Russell syndrome and Minamata disease helped the elucidation of Minamata disease as chronic methylmercury intoxication.

TABLE 14.2
Dose-Effect Relationship of Hydrogen Sulfide

Concentration (ppm)	Effect
0.25	Smell of rotten eggs
10	Irritation of mucosa, such as eye irritation
100	Olfactory fatigue, which disables the sense of smell
400	Unconsciousness, pulmonary edema, death
1,000	Instant death

Gaseous poisoning in industries was reported after the eighteenth century. Carbon monoxide (CO) poisoning is the most common form of gaseous poisoning. The mechanism of the toxicity of CO is that CO combines with hemoglobin (Hb) to form stable carboxyhemoglobin (COHb). This results in reduction of the oxygen-carrying capacity of the blood, leading to tissue hypoxia (Ilano and Raffin 1990). CO asphyxiation has been documented from ancient Rome (Morandi and Eisenbud 1980). CO was discovered scientifically in 1799, and the first CO poisoning was reported in 1842. Once, accidental acute CO poisoning in coal mines was the major cause of illness and death among workers. Acute CO poisoning is the most common gas poisoning and widely occurs among workers in a smelting furnace, boiler room, and underground parking area.

Hydrogen sulfide (H_2S) is generated by the degradation of organic compounds containing sulfur and incomplete oxidation in nature. In volcanic areas, H_2S poisoning occurs in the environment. The first H_2S poisoning in workplaces was reported in 1785. Since then, it has occured among workers in the treatment of sewage and effluent waste (Costigan 2003) and the production of pulp. The health effects of H_2S are summarized in Table 14.2. Humans can smell the odor of H_2S, similar to that of a rotten egg, at a very low level, 0.25 ppm. However, olfactory fatigue, which disables the sense of smell, occurs at 100 ppm (Costigan 2003; Hirsch and Zavala 1999). Under a poor warning system for detecting the gas, people could be exposed to more than 400 ppm H_2S and die instantly.

14.1.4 MODERN ERA: TOXICOLOGY AND PREVENTIVE MEDICINE

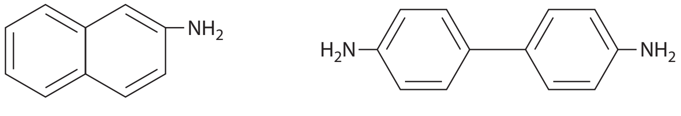
The twentieth century, especially after World War II, which was an era of occupational health problems caused by toxic substances in workplaces, was also an era for the development of toxicology and preventive medicine. Learning from severe lessons of the occurrence of occupational diseases, workers' health must come before the development of industries now. In the United States, H.G. Wiley was a central figure in the fight for the passage of the first law for pure food and drugs, and the Wiley Bill (the Pure Food and Drugs Act) was passed in Congress, and President Theodore Roosevelt signed the bill in 1906 (Wong and Tan 2009). This law is also the first countermeasure for occupational diseases caused by toxic substances. In the fields of science, animal experiments and statistics were developed in the twentieth century, and risk assessment for chemical compounds became possible. Occupational toxicology developed dramatically and became the basis for health management for workers in modern times.

14.2 CHANGING WORKPLACE ENVIRONMENT

The environment in the workplace changes with time. Once, occupational diseases occurred from exposure to toxic compounds at high levels; however, now the levels have decreased in developed countries because of preventive methods, including various regulations. The regulations include the prohibition of the production and use of toxic compounds. The prohibited toxic compounds are benzene, which was used as an organic solvent and can cause leukemia and aplastic anemia (Aksoy 1985); benzidine and 2-naphtylamine (Figure 14.2), which was used as an intermediate product for dye and can cause bladder cancer (Vineis 1994); yellow phosphorus, used for the material for friction matches and rodenticides that can cause mandibular necrosis; and several forms of asbestos, amosite, and crocidolite, used for heat insulating materials, firewalls, and car brakes, which can cause lung cancer and pleural mesothelioma (Merchant 1990).

For the chemical compounds used currently, various recommended levels in the workplace exist for the prevention of occupational diseases. The management of the environment in the workplace is one of important principles for the prevention of occupational diseases. The ACGIH (American Conference of Governmental Industrial Hygienists, Inc.) publishes threshold limited values (TLVs) as the values recommended for airborne exposure concentrations of chemical substances (ACGIH 2001). These values represent conditions under which nearly all workers are repeatedly exposed day after day without adverse health effect. ACGIH also proposes biological exposure indices (BEIs) as guidelines for biological monitoring. TLVs and BEIs are described in detail in this chapter. In addition, in the United States, the Occupational Safety and Health Administration of the Department of Labor promulgates permissible exposure limits (PELs), which are legally enforceable standards. The National Institute for Occupational Safety and Health (NIOSH) publishes recommended exposure limits (RELs).

Another important management is the improvement of the working process to reduce the exposures to chemical compounds. Wearing adequate preventive apparatus is an example for this management. The use of safer substitutes for toxic compounds is also important. Medical checkups in workplaces, which check whether adverse health effects occur among workers within early stages, are also important for management.



2-naphthylamine

benzidine

FIGURE 14.2 2-Naphthylamine and benzidine.

Even under good management, the risks of chemical toxic substances in workplaces would continue. Chronic toxicities by toxic substances at lower levels have not been elucidated yet. Thousands of novel chemical compounds are introduced in workplaces every year, and it is important to evaluate their risk by the current best scientific evidence before their use, not after the occurrence of occupational diseases. The example of the evaluation of a toxic substance before its use is the evaluation of the toxic effect of an artificial mineral fiber (Shibata et al. 2007). Lately, there are several substances with risks that are difficult to evaluate by the current best scientific evidence. Endocrine disruptors may have low-dose effects that are against dose–response relationship. Nanoparticles, which have different kinetics in bodies because of their extremely small size, go into the brain by inhalation (Takenaka et al. 2001; Kreyling et al. 2002). Nanoparticles are described in detail in this chapter. For the risk assessments of these new materials, occupational toxicology must be developed further.

14.3 THRESHOLD LIMIT VALUES

As described, TLVs are values under which nearly all workers are repeatedly exposed day after day without adverse health effects for airborne exposure (ACGIH 2001). Three types of TLVs are suggested. The time-weighted average TLV (TLV-TWA) is the time-weighted average concentration over a normal 8-hour workday and a 40-hour work week regimen. TLVs-TWA are generally applied to toxic substances that exert their effects over long periods. The short-term exposure limit TLV (TLV-STEL) is a 15-minute TWA exposure that should not be exceeded at any time during the workday even if the 8-hour TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the TLV-STEL should not be longer than 15 minutes and should not occur more than four times per day (15 × 4 = 60 minutes per day). The ceiling limit TLV (TLV-C) is the concentration that should not be exceeded during any part of the working exposures. TLV-STEL and TLV-C are applied to toxic substances that cause acute effects.

It should be noted that TLVs are not identical to the condition under which there are no risks for occupational diseases. Even if the concentration in the workplace were under the TLVs, the relation of the condition to occupational diseases would not be completely denied.

14.4 BIOLOGICAL EXPOSURE INDICES

The exposure to a chemical substance is evaluated by biological monitoring. BEIs are the values for guidelines for biological monitoring (ACGIH 2001). BEIs represent levels most likely observed in specimens collected from healthy workers who have been exposed to chemicals to the same extent as workers with inhalation exposure at the TLV.

The definition of *biological monitoring* is the evaluation of the exposure by analysis of toxic substances or their metabolites in biological materials such as urine, blood, exhaled air, and hair. For BEIs, analysis of urine, blood and exhaled air is recommended. Hair and other specimens are used in research; however, there are no BEIs for these. The examples for biological monitoring in the workplace are the

TABLE 14.3
Organic Solvents and Their Metabolites in Urine

Organic Solvent	Metabolite
Toluene	Hippuric acid
Xylene	Mandelic acid
Styrene	Mandelic acid
Tetrachloroethylene	Trichloroacetic acid, trichloroethanol
1,1,1-Trichloroethane	Trichloroacetic acid, trichloroethanol
Trichloroethane	Trichloroacetic acid, trichloroethanol
N-N-Dimethylformamide	N-Methylformamide
N-Hexane	2,5-Hexanedione

evaluations of the exposures to organic solvents. The major organic solvents and their metabolites in urine are shown in Table 14.3. For biological monitoring, a compound like an organic solvent that is rapidly metabolized should be sampled when its concentration is at the highest level, that is, at the end of the workday after two consecutive working days.

The concept of biological monitoring may be defined in a wider sense. A determination of an index in a body that is sensitively altered by a toxic compound can be an index for an exposure to a chemical. For example, although lead in the blood is used for the index for the exposure to lead, the decrease in the activity of δ-aminolevulinic acid dehydrogenase (Hemberg et al. 1970), which is inhibited by lead at the lowest level compared to the levels for other effects of lead, could be used for the index of the exposure. The use of the determination of a sensitive index in the body as biological monitoring instead of the determination of toxic compounds or their metabolites may be useful. This method would not be adequate if there is a problem of specificity. The index, such as for methhemoglobin, which is synthesized by the exposure to nitrobenzene or aniline, however, is also altered by various chemical compounds and other diseases and is not adequate for biological monitoring in a wider sense.

14.5 RESPIRATORY TOXICITY

Generally speaking, the major routes by which toxic substances gain access to the body are the gastrointestinal tract (oral exposure), lungs (inhalation) and skin (percutaneous exposure). Inhalation and percutaneous exposure are important in the workplace. There have been many occupational diseases by inhalation of toxic substances in workplaces. The major role of the respiratory system is gas exchange of oxygen and carbon dioxide, which are essential to the living body. Since fresh air is inhaled from outside, it is inevitable to have risk when exposed to toxic substances outside. Gas exchange occurs in alveoli. Adult human lungs contain 300 million alveoli, and total area of alveoli is about 60 m^2. At the area of alveoli, abundant capillaries with blood are separated from the air space by a thin layer of tissue, and the absorption of chemical compounds easily occurs there. Before reaching the lungs, irritants such as acid or base cause various airway injuries. Some of the toxicants can reach bronchioles or alveoli, and these toxicants are absorbed or stay there, also causing serious occupational respiratory diseases. The examples of the toxicants that can reach bronchioles or alveoli are nitrogen oxide, silica, and asbestos.

14.5.1 IRRITATION OF AIRWAYS AND EDEMA

Acids or bases, which are caustic or corrosive, directly impair cells and tissues and cause erosion. Sulfur dioxide binds to steam from water in the air and forms sulfuric acid mist (*mist* is a suspension of fine drops of a liquid in a gas). The mist is inhaled and causes airway injury and obstructive lung diseases. Hydrogen chloride, which is widely used as hydrochloric acid, is a gas at a normal temperature and binds to steam from water in moist air and forms the mist that causes airway injury after inhalation. Hydrofluoric acid, a halogenated hydrogen, causes chemical burns (Qureshi et al. 2002) and many deaths after inhalation in workplaces. It directly injures the airway and causes serious edema; in a serious case, the worker who inhaled died (Dote and Kono 2004).

Nitrogen dioxide is a material for nitric acid. The workers who engage in the production of nitric acid or washing by nitric acid have a risk of inhaling nitrogen dioxide. Nitrogen dioxide irritates airways and causes dyspnea. Because of relatively lower solubility in water, it reaches deep in the lung, at the level of the bronchioles and alveoli. By reaching the deep lung, it induces inflammation of tissues around the bronchioles, and pulmonary edema occurs after a latent time of several hours. Chronic exposure to nitrogen oxide causes chronic bronchiolitis and pulmonary edema.

Isocyanate-induced asthma is probably the most frequent type of occupational asthma (Deschamps et al. 1998). *Isocyanate* is a collective term that characterizes substances with a structure R-NCO. An example of R is aliphatic hydrocarbon or aromatic hydrocarbon. The representative isocyanate is toluene diisocyanate (Figure 14.3). Diisocyanates are used for reaction with polyols in the synthesis of polyurethanes. When a worker inhales isocyanate, lacrimation, nasal discharge, and cough are induced. By chronic exposure to isocyanate, the dyspnea of occupational asthma occurs (Chan-Yeung and Grzybowski 1976). The isocyanate-induced asthma is diagnosed by the symptoms, pulmonary function tests, and the determination of isocyanate in the exhaled air of workers. The symptoms occur after working hours and at night, with improvement over the weekends.

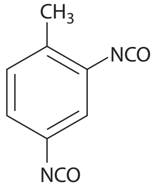


FIGURE 14.3 Toluene 2, 4-diisocyanate.

14.5.2 OCCUPATIONAL RESPIRATORY DISEASES

The most important occupational respiratory disease is pneumoconiosis (Tonori et al. 2005). Pneumoconiosis is defined as a disease of pulmonary fibrosis by inhalation of dust. By the inhalation of insoluble particles or particles with very low solubility, these particles go into the airway. The particles with a diameter of around 1 μm reach the alveoli and are deposited in the spaces or the walls of alveoli. Most of these particles are engulfed by alveolar macrophages. Although some engulfed particles in macrophages are excluded with sputum from the body, the remaining particles go into the interstitial tissue of the lung and cause increased collagen. The increase in collagen in the lung tissue becomes occupational pulmonary fibrosis, or pneumoconiosis. For the development of pulmonary fibrosis by fibers, an incomplete engulfment theory was proposed (Martina et al. 2001). According to the theory, when alveolar macrophages tried to engulf the longer-length fibers that the macrophages cannot engulf completely, the incompletely macrophage-engulfed fibers are activated. By the activation of macrophages, inflammation continues by the release of inflammatory substances, such as inflammatory cytokines, from the macrophages. Pulmonary fibrosis is promoted by the inflammation.

Among pneumoconiosis types, those induced by inorganic compounds are important in occupational medicine. These are silicosis, which is observed in workplaces with free silicate; atypical silicosis, as seen in a foundry; asbestosis, found in workplaces with asbestos; talcosis, induced by talc [$Mg_3Si_4O_{10}(OH)_2$] among talc mine millers and workers in rubber industries (Hildick-Smith 1976); agalmatolite pneumoconiosis, found among agalmatolite miners and processors for the production of a melting pot (Kishimoto et al. 1999); aluminosis, caused by aluminum or aluminum oxide in manufacture of aluminum products and abrasives; graphite pneumoconiosis, seen in those involved in the manufacture of graphite and electrodes; and carbon black pneumoconiosis, activated carbon pneumoconiosis, diatomaceous earth pneumoconiosis, and coal worker's pneumoconiosis, found among coal miners (Ross and Murray 2004). In addition, pneumoconiosis related to work itself has been reported. For example, welders' pneumoconiosis has been observed in many industries.

Among them, silicosis and asbestosis still continue to be important diseases worldwide. Silicosis is a disease produced by inhalation of one form of crystalline silica (SiO_2) at a high level (Mossman and Churg 1998; Ross and Murray 2004). Silicosis occurs after more than 5 years of exposure. Usually, it occurs after 10 to 20 years of exposure. Silicosis progresses even after the cessation of exposure. Chief complaints of silicosis are shortness of breath, palpitations, coughing, and sputum production. For the pulmonary function test, a decrease in the FEV_1 (forced expiratory volume in 1 second) is observed, and sometimes a minor decrease in total lung capacity is observed. As complications, chronic emphysema and chronic bronchitis are common manifestations of silicosis. Tuberculosis is also an important complication. Once tuberculosis develops in patients with silicosis, it is difficult to cure and causes death.

Asbestosis is defined as bilateral interstitial fibrosis of the lungs caused by the inhalation of asbestos (Mossman and Churg 1998; Ross and Murray 2004). The

International Labor Organization (ILO) defined the term *asbestos* as the fibrous form of mineral silicates belonging to rock-forming minerals of the serpentine group, that is, chrysotile (white asbestos); and of the amphibole group, that is, actinolite, amosite (brown asbestos, cummingtonite–grunerite), anthophyllite, crocidolite (blue asbestos), tremolite, or any mixture containing one or more of these. Because of their durability, fire-resistant properties, and insulating and soundproofing characteristics, asbestos was widely used for brake linings and clutch pads for cars, slate roofing, insulating board, and asbestos spray.

Asbestos-related diseases have lengthy latent periods, from 20 to 30 years; however, pleural effusions can occur within a year after the first exposure (Cugell and Kamp 2004). Pulmonary fibrosis can occur within 10 years after intense exposures (Mossman and Churg 1998), and no current treatment effectively alters the natural course of asbestosis. Therefore, use of asbestos is strictly inhibited in developed countries.

For diagnosis of asbestosis, it is important to check whether there is a history of occupational exposure. As clinical symptoms of asbestosis, dyspnea and dry cough are commonly observed. Complaints of dyspnea with auscultatory crackles on physical examination should prompt further investigation. In the advanced stages, a pulmonary function test reveals a restrictive pattern with decreased total lung capacity and vital capacity (O’Reilly et al. 2007; Ross and Murray 2004). Chest radiography typically demonstrates increased interstitial marking, especially in the lower lobes, and pleural plaques in the lungs of patients with asbestosis (Cugell and Kamp 2004; O’Reilly et al. 2007). Pleural effusion is sometimes observed. High-resolution computed tomography often shows parenchymal remodeling and tissue destruction, “honeycombing,” and pleural plaques.

Emphysema is sometimes complicated with asbestosis. Lung cancer and malignant mesothelioma can continue to occur after asbestosis. These are important causes of death in asbestos-exposed individuals. The risk of lung cancer in asbestos-exposed individuals is greatly enhanced by cigarette smoking (Ross and Murray 2004). For malignant mesothelioma, there is no effective therapy, and the median survival time for untreated persons is 9 months.

14.6 OTHER OCCUPATIONAL DISEASES CAUSED BY TOXIC SUBSTANCES

14.6.1 Metal Fume Fever

Fumes originate from heating solid substances beyond their boiling points (Vogelmeier et al. 1987), and the substances become particles again in air. Generally, these fumes are metal fumes. Metal fume fever is induced by intense inhalation of metal fumes with a particle size around 1 μm. The body temperature is sometimes increased above 39°C. In most cases, metal fume fever is due to exposure to zinc oxide fumes. Metal fumes induced by copper, magnesium, cadmium, manganese, and antimony are also reported. The reasons why zinc is a major cause of metal fume fever are considered to be as follows: Zinc is widely used for galvanization, brass plating, dyes, and electroplating (Cooper 2008). The boiling point of zinc is relatively lower compared to other metals; therefore, zinc forms fumes relatively easily. The diameters of many particles of zinc oxide fumes are around 1 μm, and the fumes reach the alveoli relatively easier compared with the larger fumes. However, the mechanism of the metal fever has not been elucidated yet.

14.6.2 FLUOROSIS

Fluorosis caused by consumption of fluoride in the groundwater in China and India has been reported (Yu 2004), and fluorosis induced by occupational exposures to gaseous fluoride in the workplaces for production of fluoride-containing substances has also been reported (Tsunoda 1985). Osteofluorosis (skeletal fluorosis) occurs among workers in an indoor air environment with high fluoride levels for 10 to 20 years. Exostosis, a spur or bony outgrowth from a bone, is observed anywhere from the vertebrae and pelvis to the long bones. Calcification occurs in the ligamentum in joints. Stiffness and gait disturbance occur by exostosis and calcification.

14.6.3 DISEASES CAUSED BY SENSITIZERS

A *sensitizer* is a substance that binds to proteins in a body following inhalation or skin contact, and the binding product leads to allergy by antigen–antibody reaction (Endo 2009). A sensitizer is an incomplete antigen (hapten). In workplaces, respiratory sensitizers and skin sensitizers induce health hazards. There are many classifications for sensitizers. In the United States, the ACGIH assigns "SEN" notations to sensitizers. From 2008, the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) has been used internationally as standard criteria for sensitizers. The GHS addresses classification of chemicals by types of hazards and proposes harmonized hazard communication elements, including labels and safety data sheets. According to the GHS, a *respiratory sensitizer* is a substance that will lead to hypersensitivity of the airways following inhalation of the substance (United Nations Economic Commissions for Europe [UNECE] 2004). A *skin sensitizer* is a substance that will lead to an allergic response following skin contact. A substance is classified as a respiratory sensitizer if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity or if there are positive results from an appropriate animal test. However, for an "appropriate" animal test, at present recognized and validated animal models for the testing of respiratory hypersensitivity are not available. A substance is classified as a skin sensitizer if there is evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons, or if there are positive results from an appropriate animal test. Since recognized and validated animal models are available for skin sensitization, evidence from animal studies is usually much more reliable than evidence from human exposure. Recognized and validated tests in animal studies include the local lymph node assay, for which mice are used; the guinea pig maximization test; and the Buehler guinea pig test.

Representative respiratory sensitizers include isocyanates, beryllium, glutaraldehyde, and phthalic anhydride (Figure 14.4). Isocyanates, as previously described, induced an allergic respiratory disturbance (Chan-Yeung 1976). Beryllium, which is used in the aerospace and computer industries (Meyer 1994) and known as the

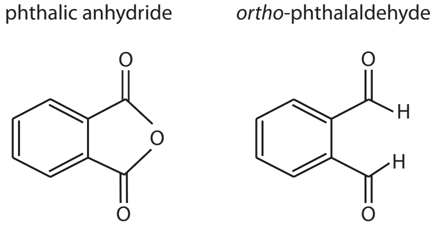


FIGURE 14.4 Phthalic anhydride and *ortho*-phthalaldehyde.

cause of chronic beryllium disease with granulomas in the lung (Rossman 1996), can directly cause inflammation of any contacted tissue, including skin and that of the respiratory tract (Meyer 1994). It also can cause chemical pneumonitis. Glutaraldehyde, which has been used as a disinfectant for endoscopy, induces asthma by acute exposure (Takigawa and Endo 2006). Because asthma induced by glutaraldehyde was reported, *ortho*-phthalaldehyde (Figure 14.4) has been used as an alternative (Fujita et al. 2006). However, a case of occupational bronchial asthma caused by *ortho*-phthalaldehyde was also reported. For hardeners for epoxy resins, acid anhydrides have been used, and the inhalation of the dust of phthalic anhydride causes chronic bronchitis and asthma.

Skin sensitizers include chromium and nickel (Minang et al. 2006). The workers involved in chromate production, chromate pigment production, and chromium plating are exposed to chromium compounds (Holmes et al. 2008). Hexavalent chromium is the most potent toxin among chromium compounds. The toxic mechanism of hexavalent chromium is considered to be the production of free radicals in the reduction from hexavalent chromium to trivalent chromium. In addition to the occupational diseases caused by hexavalent chromium that include ulceration of skin, nasal septal perforation (Williams 1998), nasal cancer, and lung cancer (Holmes et al. 2008; Norseth 1981), chromium causes allergic dermatitis (Uter et al. 1998). Nickel, which causes lung cancer (Shen and Zhang 1994), also causes allergic contact dermatitis (Minang et al. 2006).

Glutaraldehyde is a skin sensitizer (Takigawa and Endo 2006) as well as a respiratory sensitizer, and its alternative *ortho*-pthalaldehyde is also a skin sensitizer (Fujita et al. 2006) as well as a respiratory sensitizer. Urushiol, which presents as a wax on the poison ivy leaf and is used as a main component of lacquer in Japan, cause allergic contact dermatitis (Griffiths and Nickoloff 1989). Natural rubber latex is widely used for gloves for doctors, nurses, and other health care personnel. Because its proteins act as allergens, it can cause allergic contact dermatitis (Doutre 2005).

14.7 RECENT CHEMICALS OF CONCERN

14.7.1 NANOPARTICLES

The pneumoconiosis induced by particles with around a 1-μm diameter has been a public health concern. However, since nanomaterials such as carbon nanotubes, fullerene, and quantum dots have widely been used in industries, their toxic effects caused by exposure to extrasmall particles with nanolevel diameters have become new public health concerns. According to a study by Lam et al. (2004), mice that had carbon nanotubes intratracheally instilled once had induced epithelioid granulomas and interstitial inflammation in their lungs on 7 or 90 days after the instillation. It was suggested that carbon nanotubes can be more toxic to the lungs than quartz, which was used as a positive control in the study.

In previous studies, it was suggested that nanoparticles enter the brain because of their extrasmall size. Neurotoxicity is a possible consequence of nanoparticles. Takenaka et al. (2001) demonstrated that after the inhalation of ultrafine silver particles with 15-nm modal diameter, silver was detected in the brain and other organs. In a study by Kreyling et al. (2002), rats inhaled ^{192}Ir-labeled iridium particles with a 15- or 80-nm median diameter, and there was a very small but detectable fraction of ^{192}Ir-labelled iridium particles in various organs, including the brain.

Robichaud et al. (2005) assessed the relative risk associated with the production of specific nanomaterials, such as single-wall carbon nanotubes, buckyballs, one variety of quantum dots, alumoxane nanoparticles, and nano-titanium dioxide. They concluded that the relative environmental risk from manufacturing each of these materials was comparatively low in relation to other common industrial manufacturing processes.

There are difficulties in studies of the toxicities of nanomaterials. Since nanoparticles aggregate, it is difficult to expose particles with diameters at the nano level to experimental animals. Further studies are needed.

14.7.2 Rare Metals

Rare metals include metals that are used in industries, such as nickel and chromium, except for base metals, such as iron, copper, zinc, aluminum, and noble metals. Various new rare metals have been used in industries and their health effects reported. For example, the use of indium compounds in the electronics and semiconductor industry has increased. Indium oxide is often used to make thin-film transistor liquid crystal displays. Homma et al. (2003) reported a case of interstitial pneumonia in a worker who inhaled indium oxide particles. The importance of checking new materials before their use is increasing.

REVIEW QUESTIONS

1. What was the first report of chemical carcinogenicity?
2. What is the predominant protein in the urine of workers with cadmium intoxication?
3. What is the most sensitive index of lead toxicity?
4. Why does instant death occur in humans after exposure to hydrogen sulfide at very high levels despite the fact that humans can detect the gas at a very low level?
5. What are the three types of TLVs?
6. What are the guideline values for biological monitoring set by the ACGIH?

7. What are the important causes of death in asbestos-exposed individuals?
8. Which metal is the most common cause of metal fume fever?
9. What are the definitions of respiratory and skin sensitizers?

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15 Endocrine Disruption

15.1 INTRODUCTION

Endocrine disruption is one of the most pressing environmental issues facing environmental toxicology. It is generally perceived that exposure to certain anthropogenic chemicals that can interact with and disrupt the endocrine system may cause some form of malfunction and ultimately pose serious health problems in humans, wildlife, fisheries, or their progenies (Pickering and Sumpter 2003; Fossi 2001). Chemicals that can induce endocrine disruption are called *endocrine disrupters* (EDs) or *endocrine-disrupting chemicals* (EDCs).

By a broad definition, EDs are exogenous chemical agents that interfere with the synthesis, secretion, transport, binding, action, metabolism, or elimination of natural hormones (U.S. Environmental Protection Agency [USEPA] 1997). They are a group of chemicals with diverse structures and include those widely used in the past in industry and agriculture, such as organochlorine pesticides and polychlorinated biphenyl (PCBs), and those currently used, such as plasticizers and surfactants. Many of the known EDs are estrogenic, affecting reproductive functions in particular. In addition, certain EDs are capable of mimicking the actions of progesterone, whereas others are antiestrogenic or capable of acting on the thyroid. Because of the persistent and lipophilic nature of most xenobiotic estrogens and their metabolites, many EDs bioaccumulate and biomagnify, potentially inducing adverse effects in living organisms.

15.2 REVIEW OF HORMONAL FUNCTION

Before discussing more specific aspects of EDs, it is important to briefly review how hormones function in the body. Hormones are specific organic substances produced by the endocrine system. They are transported by body fluids and produce specific effects on the activities of cells remote from their points of origin. The hormones that have received wide attention in recent years are estrogen and androgens. Most hormones are comprised of steroid and are generally referred to as steroid hormones. These hormones are formed from cholesterol through a complex biochemical pathway. Figure 15.1 shows the main steps involved in the pathway.

Estrogen is one of the steroid hormones. It is a sex hormone, as estradiol, estriol, and estrone. Estrogen is generally considered a female hormone, but it is produced in both males and females, although the quantities produced in females are much greater than those in males (Eubanks 1997). Estrogen produced especially in the ovaries is characterized by its ability to promote estrus and stimulate the development of secondary sex characteristics in the female. Estrogen also refers to a substance occurring naturally in plants or made synthetically (as DES [diethylstilbestrol]) that has similar biological activity. Androgen, also a sex hormone (as androsterone and

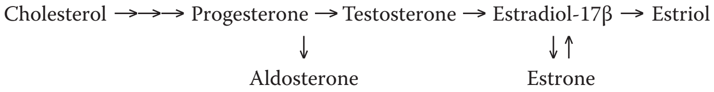


FIGURE 15.1 Main steps involved in steroid hormone synthesis.

testosterone), is produced especially in the testes and adrenal cortex and is usually characterized by its ability to stimulate the development of sex characteristics in the male. Androgen also refers to a synthetic compound having similar biological activity. Figure 15.2 shows the structure of some steroid hormones.

The production of hormones is regulated by a complicated negative-feedback pathway that is turned on and off in response to changes in hormone levels. When hormone production peaks, the hormone acts as an inhibitor and causes the pathway leading to the production of the substance to shut down (Eubanks 1997). Once hormones are produced, they travel through the bloodstream to target cells, where they attach to the receptor protein, forming a hormone-receptor complex that then enters the cell nucleus and binds to the DNA. Transport of the hormone and hormone-receptor complex to DNA of a cell can occur in one of three ways, depending on the type of hormone. A hormone may bind to a receptor protein that carries it to the cytoplasm of the cell. A hormone may move directly into the nucleus, where it binds to its receptor protein and initiates transcription of messenger RNA. With androgens and estrogens, their lipophilicity should facilitate their passage across the cell membranes to the cytoplasm. In the case of a hydrophilic hormone such as a peptide that cannot pass through the plasma membrane, the molecule binds to receptor proteins on the surface of the cell (Eubanks 1997). Binding of the protein receptor causes a change in the shape of the protein and induces a series of events in the cytoplasm. For instance, the protein receptor is often composed of several subunits. As it forms a complex with an incoming hormone, the receptor may release some of its subunits (Figure 15.3).

Chemicals may be activated or deactivated as the signal is passed from one molecular receptor to another. Hormones initiate cellular and physiological changes by altering transcription of specific genes within the cell nucleus. In some cases, the hormone-receptor complex may inhibit transcription. It should be pointed out that only small amounts of hormones are necessary for inducing vital cellular and physiological responses. An organism will therefore be sensitive to any changes in the amount of hormone that is produced or that enters the cytoplasm. Furthermore, the interaction between the hormone and the receptor is precise and constitutes the reception of a chemical message by a particular cell. The reaction to the interaction of the hormone and the receptor is specific to the type of cells involved (Landis and Yu 2004). Figure 15.3 diagrams the interaction between a hormone and its protein receptor in a cell.

15.3 CHARACTERISTICS OF ENDOCRINE DISRUPTORS

A variety of EDs has been identified. They include pesticides (including herbicides and fungicides), plasticizers, surfactants, organometals, halogenated polyaromatic

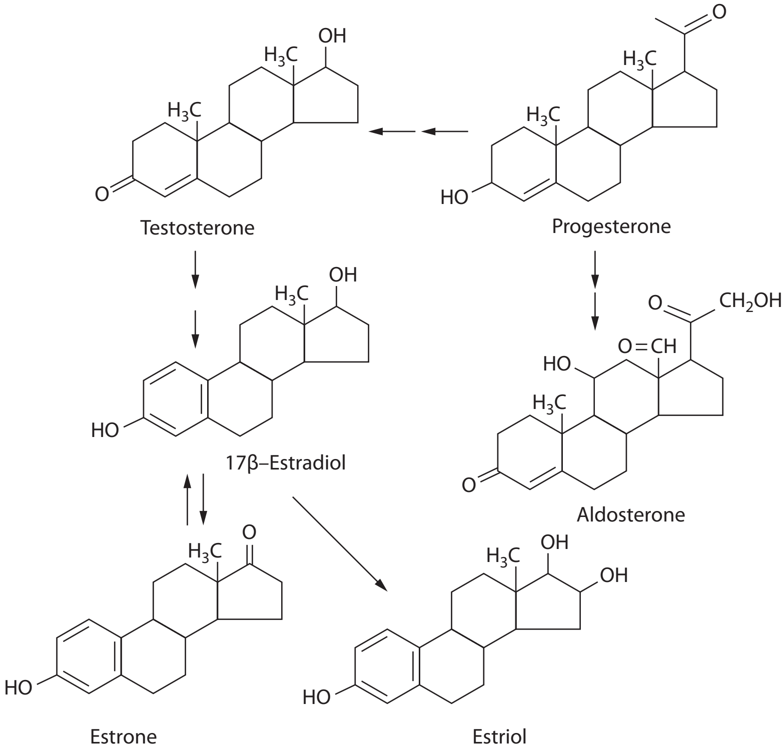

Testosterone
Progesterone
17β–Estradiol
Aldosterone
Estrone
Estriol

FIGURE 15.2 Structures of some steroid hormones.

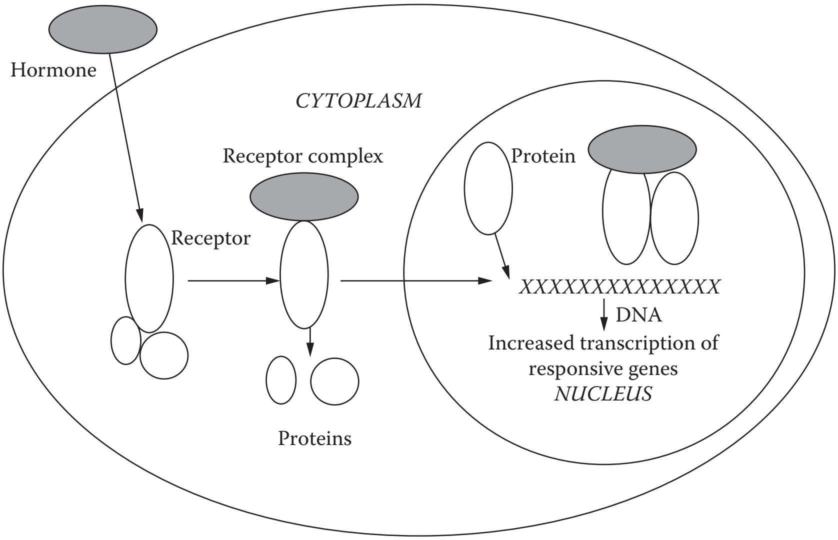

Hormone
CYTOPLASM
Receptor complex
Receptor
Protein
XXXXXXXXXXXXXX
DNA
Increased transcription of responsive genes
NUCLEUS
Proteins

FIGURE 15.3 Regulatory role of steroid hormone.

TABLE 15.1
Examples of Endocrine-Disrupting and Estrogen-like Pesticides

Endocrine-Disrupting Pesticides	Estrogen-Like Pesticides
Herbicides	
2,4-D	Atrazine
2,4,5-T	
Alachlor	
Amitrole	
Atrazine	
Metribuzin	
Nitrofen	
Triflurafin	
Insecticides	
i-Hexachlorocyclohexane	i-Hexachlorocyclohexane
Carbaryl	Kepone
Chlordane	1-Hydroxychlordane
DDT and its metabolites	*p,p′*-DDT
Endosulfan	*o,p′*-DDT
Heptachlor	*o,p′*-DDE
Heptachlor-epoxide	*p,p′*-DDE
Lindane	DDT
Methomyl	DDE
Methoxychlor	Endosulfan
Mirex	Heptachlor
Oxychlordane	Methoxychlor
Parathion	Toxaphene
Synthetic pyrethroid	
Toxaphene	
Other	
Nonylphynol (NP)	

Source: Adapted from *Encyclopedia Britannica* online.

hydrocarbons, and phytoestrogens. Representative examples of items in these categories are presented in Table 15.1. An important characteristic of environmental hormones is the lack of structural similarity with the estrogen itself. Kepone (Figure 15.4), for example, does not have a structural resemblance to 17β-estradiol (Figure 15.2), a representative estrogen, but manifests a potent endocrine-disrupting effect. Second, unlike many other types of environmental pollutants, environmental hormones do not alter genes themselves but may change the way they are expressed (McLachlan and Arnold 1996).

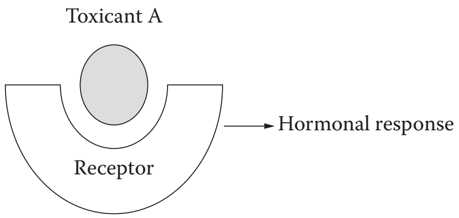
Exposure to environmental EDs has been associated with a variety of abnormalities in wildlife and fish. Some examples include abnormal thyroid function in birds and fish; decreased fertility in birds, fish, and mammals; decreased hatching success in fish and birds; demasculinization of male fish and birds and mammals; defeminization and masculinization of female fish, gastropods, and birds; and alteration of immune function in birds and mammals.

As mentioned, hormone-receptor interaction is quite specific. The manifested specificity is thought to result from two basic factors: the conformation of the receptor, and the three-dimensional structure of the xenobiotic and its resemblance to a natural ligand. For instance, studies were carried out to test the inhibitory effect of DDT and DDD isomers on the binding of tritiated estradiol ([^{3}H]-17β-estradiol) to alligator estrogen receptor. Results showed that both *o,p′*-DDT and *o,p′*-DDD manifested a potent inhibitory effect, but the close isomers *p,p′*-DDT and *p,p′*-DDD did not (Landis and Yu 2004).

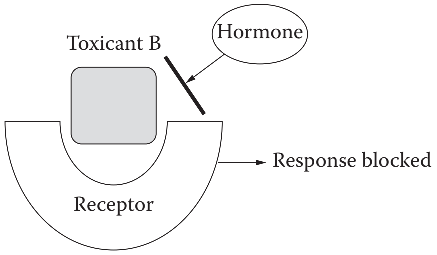
Three characteristics are associated with the toxicity of EDs: (a) their high lipophilicity, which means the EDs can thus accumulate in lipid-rich cellular components, such as the membranes; (b) their ability to irreversibly bind to macromolecules such as DNA; and (c) their ability to reversibly react at specific sites of the receptors and enzymes. Presumably, the overall toxicity of EDs is the result of the combined effect of these factors (Landis and Yu 2004).

15.4 MODE OF ACTION

Toxicants that are EDs are known to act in several basic ways. First, the toxicant may mimic the natural hormone, cause a structural change of the receptor, and initiate a response (Figure 15.4a). Toxicity may arise from an excess of hormone-producing gene products or from inhibition of transcription at inappropriate times.



(a) Hormonal mimic



(b) Hormonal block

FIGURE 15.4 Proposed mechanisms of the actions of (a) hormone mimic and (b) hormonal block.

As a result, males, for example, may become feminized in the presence of an estrogen mimic. Second, the xenobiotic may bind to a hormone receptor, inducing changes in the transcription of messenger RNA, followed by altered gene expression. By binding to hormone receptors, EDs can block the action of a natural hormone, prohibiting it from binding to the receptor. In this case, the xenobiotic may bind to the active site of the receptor and prevent the natural hormone from attaching to the active site. In this way, the xenobiotic not only occupies the active site of the protein receptor but also prevents the conformational changes necessary for correct hormonal response from occurring (Figure 15.4b). Xenobiotics that act this way are called *estrogen antagonists*. An example of the outcome of such alteration is masculinization of the female (Landis and Yu 2004). As noted in Chapter 13, an endocrine disrupter such as 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) may interact with an aryl hydrocarbon (Ah) receptor, disrupting natural hormone function in the cell.

Other mechanisms include alteration in hormone synthesis, metabolism, and transport as well as effects mediated through changes in the hypothalamic-pituitary-gonadal axis (Landrigan et al. 2003). For example, a report indicated that some EDs may cause endocrine disruption by enhancing endogenous estrogen through inhibition of the enzymes involved in the biotransformation and inactivation of the estrogen (Figure 15.5) (Song 2001). According to the report, certain hydroxylated PCBs (OH-PCB) are potent inhibitors of estrogen sulfotransferase (EST) in humans. EST is a cytosolic enzyme and catalyzes the conversion of estrogen such as 17β-estradiol into 3-sulfonyl estradiol (Figure 15.6). The conversion results in inactivation of the hormone. The sulfonyl group needed for the conversion is provided by 3′-phosphoadenosine-5′-phosphosulfate (PAPS). The enzyme (EST) has a high affinity for estrogens and is expressed in several estrogen target tissues, including the male and female reproductive systems (Landrigan et al. 2003).

The observation that EST serves as a target for OH-PCB has a significant implication. The finding demonstrates that an environmental chemical does not have to interact with the estrogen receptor itself to cause endocrine disruption. In other words, a

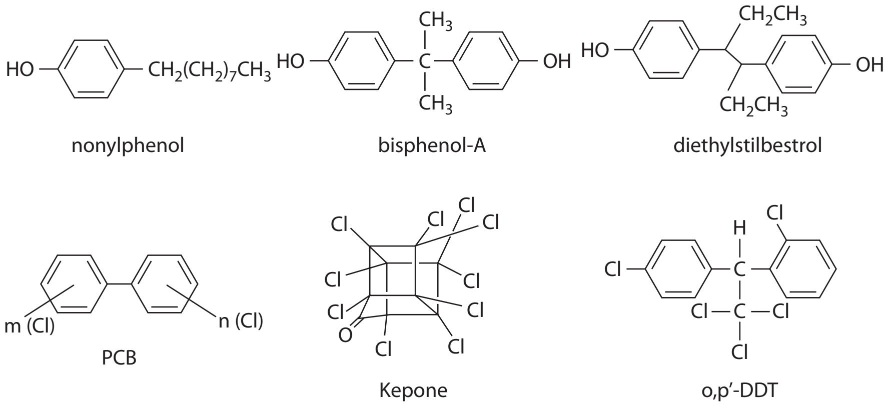


FIGURE 15.5 Structures of several endocrine disrupters.

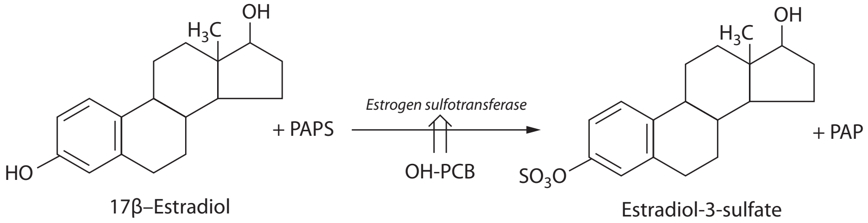


FIGURE 15.6 Hydroxylated PCB (OH-PCB) inhibits the catabolism of estradiol by estrogen sulfotransferase. PAPS, 3′-phosoho-adenosine-5′phosphosulfate. (Adapted from Song, W.-C. Biochemistry and reproductive endocrinology of estrogen sulfotransferase. *Ann. N. Y. Acad. Sci.* 948, 43, 2001.)

xenobiotic such as OH-PCB can inhibit the enzyme EST in estrogen target tissues and induce an increase in endogenous estrogen activity and toxicity (Landrigan et al. 2003). The finding thus provides a new paradigm in explaining the endocrine-disrupting potential of environmental chemicals that have low or no binding affinities for steroid hormone receptors (Song 2001).

According to some studies, estrogens throw cold water on fish courtship behavior (Hileman 1994; *Environ. Sci. Technol.* 2010). Synthetic estrogen in birth control pills that find their way into the environment via wastewater can produce subtle changes in the female zebrafish's courting behavior, which could alter the genetic makeup of the fish over time. Since the beginning of the twenty-first century scientists have reported the effects of these hormone pollutants on male fish development, such as testes feminization. But, Charles R. Tyler of the University of Exeter, England, and colleagues wanted to determine whether estrogens could also affect female fish. The researchers exposed 20-day-old zebrafish to ethinyl–estradiol for 40 days and then compared the actions of the fish to those of unexposed fish when they all reached adulthood. Estrogen-exposed females abandoned normal courting behaviors, such as chasing suitors. By shunning interested males, these affected females produced 70% fewer viable embryos than unexposed females. The estrogen-exposed female fish also mated less often with dominant males and more often with less-virile fish. Eventually, genes from these less-dominant males could become more common, the researchers said, which could thwart the ability of the population to adapt to environmental changes (Harris et al. 2011).

15.5 EXAMPLES OF ENDOCRINE DISRUPTION

The widespread presence of environmental estrogens and the possibility that their degradation products will persist in the environment, coupled with the potential for inadvertent exposure of humans and wildlife to EDs, raise significant safety issues. Although most of the pesticides show weak endocrine-disrupting activity, some researchers consider it still possible that chronic exposure to those pesticides may lead to toxicity. A large number of findings have been reported in recent years concerning xenobiotics causing endocrine disruption. Several examples are briefly reviewed in the following sections.

15.5.1 Induction of Developmental Toxicity

Induction of developmental toxicity is viewed by many researchers as the most important action of EDs. The chemical classes that have been shown to induce developmental toxicity include pesticides, herbicides, fungicides, plasticizers, surfactants, and halogenated polyaromatic hydrocarbons. For example, a specific ratio of estrogen to androgens is necessary for sexual differentiation. Endocrine disrupters can perturb the specific ratio of estrogen to androgens in the developing fetus of humans and animals (McLachlan and Arnold 1996; Landrigan et al. 2003; Song 2001). This perturbation may result in offspring with two sets of partially developed sexual organs, termed *intersex,* or with a single set that is improperly or incompletely developed (Fossi 2001).

The Lake Apopka alligator episode has provided a unique example, suggesting that environmental toxicants can potentially induce developmental toxicology in wildlife (McLachlan and Arnold 1996; Ladrigan et al. 2003; Song 2001). Lake Apopka, in central Florida, is situated in an area adjacent to a chemical plant. The area became contaminated with high levels of DDT and its metabolites, which leaked from the chemical plant between 1970 and 1980. Subsequent comparative studies showed that male juvenile alligators from Lake Apopka had significantly smaller reproductive organs as well as lower concentrations of plasma testosterone than alligators from several other less-polluted lakes. Although these findings could not be linked quantitatively to current pesticide levels in Lake Apopka, it was suggested that the observations could reflect past exposures of young alligators to chlorinated hydrocarbon pesticides (Landrigan et al. 2003).

15.5.2 Estrogen Mimics

Estrogen mimics are a diverse group of chemicals that have no obvious structural similarity. Examples of estrogen mimics include DDT, DDE, kepone, dieldrin, dicofol, PCBs, and methoxychlor. These chemicals have several characteristics, such as persistency, high lipophilicity, and accumulation in adipose tissue of animals and humans over a long period of time. A majority of these chemicals appear to attach themselves to estrogen receptors and mimic the action of the body's natural estrogen or to block the action of the body's natural estrogen, as mentioned previously. In addition, estrogen mimics may interfere with the normal metabolism of estrogen in the body.

Most of the estrogen mimics can cross the placental barrier and so pass from the mother to the developing fetus. It is important to note that the amount of natural estrogen in the mother is usually much greater than the amount of estrogen mimics. However, because most of the sex-homone-binding protein in the blood is used in binding the natural estrogen, it is thus unable to bind estrogen mimics, leading to increases in the effective dose of the mimics and thus their toxicity (Landis and Yu 2004; Hileman 1994).

15.5.3 Induction of Sterility

Many reports have indicated that sperm counts in men have decreased about 50% worldwide since 1940, and that environmental estrogens are suspected of being involved. A significant decrease in male sperm counts coincides with increased use of estrogenic chemicals since about 1960 (McLachlan and Arnold 1996; Hileman 1994). For example, a spill of kepone, one of the cyclodienes (Figure 15.5), occurred in 1975. The spill resulted in a decreased sperm count in men exposed to the chemical. Subsequent studies showed that kepone, which does not have a structural similarity with natural estrogen, is indeed a weak estrogen.

According to a news media report, scientists at King's College in London have reported a direct link between the fertilizing ability of sperm and the presence of endocrine disrupters, including nonylphenol (Figure 15.5), in the environment. The researchers indicated that their study with mice was the first to provide both indirect and direct evidence that environmental estrogens significantly affect sperm fertilizing ability. According to the researchers, the estrogenic effect identified in the mouse sperm will be replicated in humans.

DES (Figure 15.5) exposure has been shown to induce malformations and adverse functional alterations of the brain and male and female reproductive tracts. In experimental animals, exposure to higher levels of DES (10 to 100 μg/kg) resulted in total sterility of female offspring. This was due in part to structural abnormalities of the oviduct, uterus, cervix, and vagina and to depletion and abnormalities of ovarian follicles.

Newborn rats treated with DES (10 μg/animal) postnatally on alternate days from day 2 to day 12 were shown to delay the establishment of the blood-testis barrier for several weeks. In addition, the diameter of the seminiferous tubule in DES-treated rats was one-half that of the control animals. The mechanism involved in the observed delay is not clear (Toyama 2001).

The impact of EDs on humans was demonstrated first by the observation of DES-induced cancer in young women. Eight cases of clear cell adenocarcinoma (CCA) of the vagina occurred in women who had been exposed to DES in utero one to two decades earlier. DES is a synthetic estrogen and was prescribed to pregnant women in the 1950s and 1960s to prevent miscarriage. According to a report by the National Research Council, more than 300 cases of CCA have been shown with the same health problem caused by in utero exposure to DES. Reproductive tract abnormalities were observed in males exposed to DES in utero. It is interesting to note that no abnormalities were observed in the pregnant women who had received DES (Landrigan et al. 2003).

15.5.4 ANTIANDROGENS

Chemicals that can bind to the androgen receptor without activating it and simultaneously prevent binding of true androgens are called *antiandrogens*. Principal manifestations of developmental exposure to an antiandrogen are generally restricted to males and include hypospadias (an abnormality of the penis in which the urethra opens on the undersurface), retained nipples, reduced testes and accessory sex gland weights, and decreased sperm production. There are indications that birth defects in the male reproductive tract have increased over the past several decades. Examples of antiandrogens are the fungicide vinclozolin and the DDT metabolite *p,p′*-DDE, *o,p′*-DDT has weak estrogenic activity (USEPA 1997).

15.5.5 INDUCTION OF IMPOSEX

Imposex is a condition in which females develop part of the male reproductive system, including a penis and a vas deferens. This effectively prevents animals from reproduction. For example, tributylitin (TBT) compounds have caused the disappearance or reduction of the dog-whelk snail along the British coast. Marine snails in the Northeast Pacific have been shown with signs of imposex caused by TBT pollution (Bright and Ellis 1990; Howell et al. 1980). Fish near sewage treatment plants in the United Kingdom develop hermaphroditic characteristics, thought to be caused by the widespread use of contraceptive pills and the subsequent release of ethynylestradiol (via sewage treatment plants). Earlier studies found that female mosquito fish downstream from pulp and paper mills in Florida were masculinized and developed the male sex organ (Bright and Ellis 1990).

The precise nature of imposex as a manifestation of endocrine disruption is still the subject of discussion but is believed to involve the suppression of the enzyme aromatase. Aromatase catalyzes the conversion of androgens to estrogens. The net effect of the suppression of aromatase is a shift in the hormonal balance of these animals to androgens.

15.5.6 HYPOTHYROIDISM

The thyroid gland produces thyroid hormone, which is responsible for basal metabolism. Hypothyroidism refers to deficient activity of the thyroid gland or a resultant abnormal state marked by lowered metabolic rate and general loss of vigor. Hypothyroidism thus causes growth retardation, cognitive deficits, delayed eye opening, hyperactivity, and auditory defects in rodents. PCBs may act at several sites to lower thyroid hormone levels during development and cause body weight and auditory deficits (Bright and Ellis 1990).

15.5.7 CHANGING BEHAVIOR

Studies indicated that estrogens may throw cold water on fish courtship behavior (Hileman, 1994; *Environ. Sci. Technol.* 2010). Synthetic estrogen in birth control pills that find their way into the environment via wastewater can produce subtle changes in the female zebrafish's courting behavior, which could alter the genetic makeup of the fish over time. Over the past decade, scientists have reported the effects of these hormone pollutants on male fish development, such as testes feminization.

15.6 HORMONAL CANCERS

15.6.1 INTRODUCTION

Endocrine disrupters are suspected of causing various types of human cancer, including breast cancer, prostate cancer, and testicular cancer. Over the past 50 years, the incidence of prostate cancer in some countries has doubled, while that of testicular cancer has tripled. Similarly, it has been shown that, since 1940, the incidence of female breast cancer has risen in Western Europe and the United States (Hileman 1994). A number of studies have shown the presence of residues of DDT and other organochlorine pesticides in human breast milk and adipose tissue. Exposure to these pesticides has been implicated in breast cancer risk.

After increasing from 1994 to 1999, the female breast cancer incidence rate decreased from 1999 to 2006 by 2.0% per year. An estimated 39,840 new cases of invasive breast cancer were expected to occur among women in the United States during 2010. Breast cancer ranks second as a cause of cancer death in women (after lung cancer). Death rates for breast cancer have steadily decreased in women since 1990, with larger decreases in women younger than 50 (a decrease of 3.2% per year) than in those 50 and older (2.0% per year) (American Cancer Society 2010).

An estimated 60–70% of human breast cancers are associated with sex hormone exposure. Approximately 60% of all breast cancer patients have hormone-dependent breast cancer, which contains estrogen receptors and requires estrogen for tumor growth (Bright and Ellis 1990). The possible roles of estrogens in the development of breast cancer are still unclear.

DES is a potent ED. It has been shown to be a transplacental carcinogen, that is, a chemical, which when given to the mother, causes cancer in her daughter. As mentioned, DES was associated with vaginal cancer in some of the adolescent daughters of women who had taken the synthetic estrogen to prevent miscarriage. In addition, it brought about cellular changes in the vagina or Fallopian tubes of female offspring, as well as structural changes in the uterus. Thus, the synthetic estrogens are capable of affecting the development of the reproductive system and subsequent adult health.

Another observation is the increase in the prevalence of endometriosis, the growth outside the uterus of cells that normally line the uterus. Endometriosis was previously a rare condition, but reportedly it now afflicts 5 million American women. This is a painful disease that affects women in their reproductive years, frequently leading to infertility. The USEPA has reported that PCBs may be involved in the induction of endometriosis (McLachlan and Arnold 1996; Hileman 1994).

15.6.2 Hormonal Cancers in Farmers

Several epidemiological studies have shown that cancer risks among farmers are increased compared with those among the general population (Pearce and Reif 1990; Blair and Zahm 1991). The most suspected agents in this trend are pesticides, but there is a lack of data that would show individual agricultural chemicals involved in the development of cancers among farmers. Most of the studies that show an association between pesticide exposure and cancer have been conducted among pesticide applicators or workers in pesticide-manufacturing plants. For example, in a retrospective cohort epidemiological study of Canadian farmers linked to the Canadian National Mortality Database, a weak but statistically significant association between acres sprayed with herbicides and prostate cancer deaths was found (USEPA 1997). In a 30-year follow-up study of coke oven workers, an association of coke oven emissions with significant excess mortality from cancer of the prostate has been observed.

Also, in several epidemiological and animal studies, there is some evidence for a role of the heavy metal cadmium in prostate cancer etiology (USEPA 1997).

In addition to cancer of the prostate, other types of cancers that have been reported to be associated with farmers include cancers of the testicle, ovary, breast, thyroid, and endometrium. However, many types of exposure are involved in farming, making it difficult to find out which exposure is associated with cancer. Obviously, further studies are needed for clarifying the issue. It is encouraging to note that the U.S. Department of Health and Human Services and Department of Agriculture have allocated funds for an extensive study on the relationship between farmers and cancers.

15.6.3 The Toxic Substance Control Act

Reproductive problems associated with human exposure to toxic chemicals are on the rise in the United States, according to a report from the nonprofit Center for American Progress (CAP) (American Chemical Society 2009). The director of regulatory and information policy at CAP showed that since 1979 a 30% increase in the number of chemicals registered for commercial use and rising numbers of fertility problems, preterm births, and birth defects have occurred. In particular, the report linked two plastics chemicals—phthalates and bisphenol A—and polybrominated diphenyl to reproductive health effects. The CAP director urged Congress to act to reform the Toxic Substance Control Act (TSCA), saying "Regulatory agencies lack the authority and capacity to adequately evaluate safety and set strong standards against dangerous chemicals." The American Chemistry Council, which represents the largest U.S. chemical manufacturers, testified in front of Congress early in 2010 that it supports modernizing the TSCA to enhance public confidence in the federal chemical management system (American Chemical Society 2009).

15.7 TESTING ESTROGENICITY

There has been an increasing concern about endocrine disruption since the 1990s. Many synthetic chemicals, such as alkylphenol polyethoxylates (APEs), PCBs, DES, and ethinylestradiol (EE_2), have been released from sewage treatment plants, industrial factories, and pulp mills into aquatic environments. Some of these chemicals have estrogenic potency acting through specific estrogen receptors and mimicking estrogenic actions. They are called *environmental estrogens*. In addition, considerable amounts of natural estrogens (e.g., estrone, estradiol, and estriol) originating from human and agricultural animals are also released into aquatic environments. The disruption of hormonal balance in an animal may disrupt development, sex determination, growth, gonadal development, fertility, spawning timing, or reproductive success. It is therefore essential to determine the degree of contamination of an environment by those chemicals and their impact on animals inhabiting this environment (Purdom et al. 1994).

A number of methods have been developed and used for studying the presence and action of EDs. One of these methods is the use of vitellogenin (Vg). Vitellogenin is a typical female protein and is the complex phospholipoglycoprotein precursor of egg yolk synthesized by the liver in response to estrogen stimulation. It has drawn much attention as a biomarker of fish exposure to environmental estrogens. It is incorporated by growing oocytes via the bloodstream and cleaved into three major yolk proteins. Very little Vg can be detected in males and juvenile fish. Induction of this typically female protein in males has been widely used as a sensitive biomarker of estrogenic effects (Fossi 2001). Vg can be measured using enzyme-linked immunosorbent assays (ELISAs) or radioimmunoassay (RIA) techniques.

The method has been widely used in many countries in the study of endocrine disruption. For example, high induction of Vg was detected in adult Mediterranean male swordfish, suggesting that these species are exposed to high toxicological risk in the Mediterranean (Sumpter and Jobling 1995). Similarly, a study, the Endocrine Disruption in the Marine Environment (EDMAR) project, and an earlier similar project have been conducted in the United Kingdom since 1996. The results from these studies showed that plasma Vg concentrations in male flounder have remained elevated in several U.K. estuaries throughout the period covered in the study (Kirby et al. 2004).

Vitellogenin is incorporated by growing oocytes via the bloodstream and cleaved into three major yolk proteins. Male or immature fish usually have no or little Vg in their circulation. However, when male or immature fish are exposed to exogenous estrogen, Vg is induced in their circulation and is therefore a good indicator of environmental estrogens. A pioneering work was conducted by Purdom et al. (1994), who placed cages containing male rainbow trout (*Oncorhynchus mykiss*) in effluent from sewage treatment plants in the United Kingdom. They demonstrated that Vg was induced by effluent from the sewage treatment plants. The incidence of endocrine disruption was further extended to other inland rivers in the United States. Subsequently, many studies have used fish Vg as a biomarker of environmental estrogens.

Among many fish species, the common carp (*Cyprinus carpio*) is one of the most frequently used test fish for field survey because of its wide distribution and ability to tolerate polluted environments. In Japan, the first survey for possible environmental disruption in a metropolitan river system, the Tama River, was carried out in 1997. In this survey, Vg was detected in male carp caught downstream of effluent from a sewage treatment plant, and testis abnormality was histologically found (Blair and Zahm 1991). The report led the Japanese government to initiate extensive surveys to evaluate the current state of endocrine disruption in Japan.

More recently, Hara et al. (2007) studied both male and female carp captured at points of effluent discharge from a sewage treatment plant connected to a river in Japan, where estrogenic compounds were later detected. The presence of Vg in their circulation was then examined. Vg was detected in both male and female carp at the milligram/milliliter level, suggesting that estrogens such as estrone and estradiol were sufficiently high to induce Vg in male carp inhabiting this area.

REVIEW QUESTIONS

1. What is a hormone? What is its function?
2. What are the differences between androgens and estrogens?
3. What is the function of androgens? Of estrogens?

4. Define *endocrine disrupters*.
5. What are the general characteristics of endocrine disrupters?
6. Explain the basic mechanisms associated with the toxicity of EDs.
7. What is meant by hormonal mimics?
8. What is meant by imposex?
9. Explain the toxicity of DES.
10. What is vitellogenin?
11. How is vitellogenin used in laboratory and field studies?

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16 Mutagenic Pollutants

16.1 INTRODUCTION

Mutation is a process wherein the hereditary constitution of a cell is altered, ultimately leading to a genetically altered population of cells or organisms. The agent that causes mutation is called a *mutagen*. Although mutations can occur in the RNA of viruses and the DNA of cytoplasmic organelles, the mutations of greatest interest occur within genes in the nucleus of the cell.

The human body is estimated to contain more than 10 trillion cells, and at some stage in its life cycle, each cell contains a full complement of the genes needed by the entire organism. Genes, composed of DNA in the nucleus, are clustered together in chromosomes. In the chromosomes of all but the most primitive organisms, DNA is combined with protein. DNA, the molecular basis of heredity in higher organisms, is made up of a double helix held together by hydrogen bonds between purine and pyrimidine bases, that is, between adenine (A) and thymine (T) and between guanine (G) and cytosine (C). Figure 16.1 shows the structures of the five bases in DNA and RNA, and the pairing of bases in DNA is shown in Figure 16.2. The highly specific complimentarity of these bases enables DNA to act as a template for its replication by DNA polymerases, as well as the synthesis of RNA transcripts by RNA polymerases. For the information contained in DNA to be biologically expressed, the sequence of the nucleotides of a gene is converted to the sequence of amino acids in a protein. The amino acid sequence determines the enzymatic and structural properties of the protein thus formed.

DNA clearly plays a pivotal role in the expression and perpetuation of life. However, it is also a critical target for the action of many mutagenic environmental chemicals; lesions in DNA may occur through the action of physical or chemical agents found in the environment. Occurrence of mutation, however, depends on the nature of the initial lesion and the response of cells to the DNA damage. If the damage is intermediate, the mutations resulting from it may be of immediate concern because mutations are implicated in pathogenesis of many inherited, somatic human diseases. However, if the damage is severe enough it can interfere with essential functioning of DNA and lead to the death of cells.

16.2 TYPES OF MUTATION

Mutations are often divided into two broad categories: chromosomal aberration and gene mutation. *Chromosomal mutation* refers to mutation that is cytologically visible, whereas gene mutation means one that occurs at the submicroscopic level and is cytologically invisible.

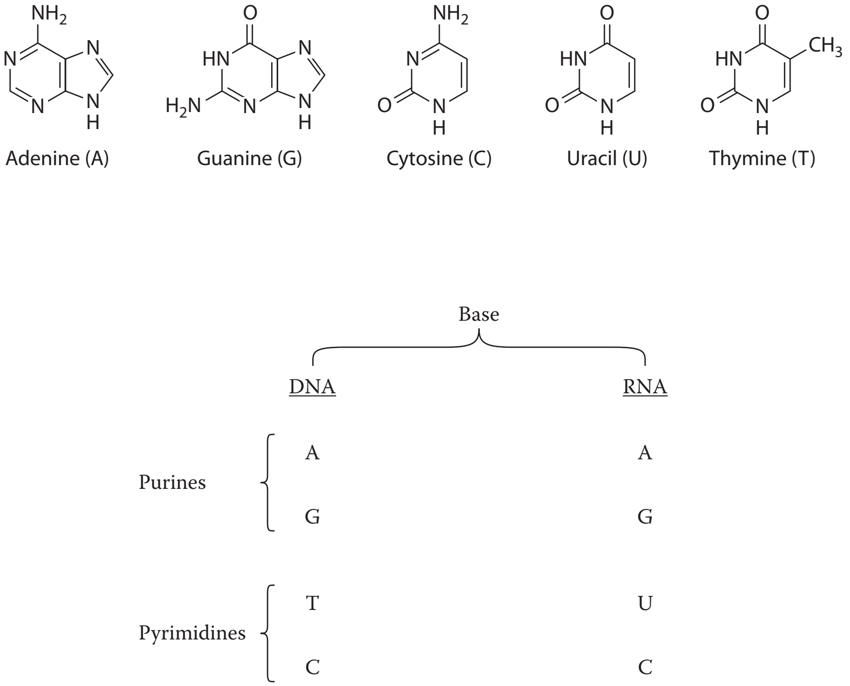

Adenine (A)
Guanine (G)
Cytosine (C)
Uracil (U)
Thymine (T)
Base
DNA
RNA
Purines
A
A
G
G
Pyrimidines
T
U
C
C

FIGURE 16.1 Structures of bases in nucleic acids.

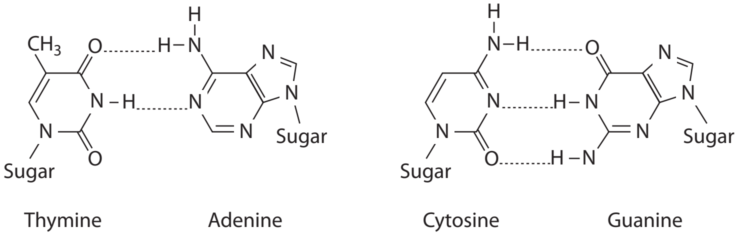

Thymine
Adenine
Cytosine
Guanine
Sugar
Sugar
Sugar
Sugar

FIGURE 16.2 Pairing of bases in DNA.

16.2.1 CHROMOSOMAL ABERRATIONS

A human cell normally has 23 pairs of autosomal chromosomes and a pair of sex chromosomes. In chromosomal aberration, mutation produces either a change in the number of chromosomes or a change in the structure of individual chromosomes. Changes that involve entire sets of chromosomes are called *euploidy*, whereas variations that involve only single chromosomes within a set are called *aneuploidy*. Alteration in chromosomal structure occurs when the chromosomes fracture and the broken ends rejoin in new combinations. Major structural changes in chromosomes include *deletions, duplications, inversions,* and *translocations.* In deletion, a portion of a chromosome is lost; for instance, in a chromosome ABCDE, the portion C is lost, resulting in ABDE, whereas in duplication, an additional copy of a portion C of the chromosome is inserted, becoming ABCCDE. Deletions and duplications both upset the metabolic balance of an organism by altering the amount of gene product that is formed. In an inversion, the order of genes on a chromosome is reversed in one area, for example, ABCDE becomes ACBDE. If a broken portion of a chromosome attaches itself to a second chromosome, it is termed a translocation, for example, ABCDE → ABDE + C (a broken portion), and C attaches itself to a second chromosome ABCD to become ABCDC. Because the position of a gene affects its regulation and activity, both inversions and translocations may be detrimental.

16.2.2 Gene Mutations

In a gene mutation, which cannot be observed microscopically, an alteration occurs in the nucleotide sequence of a gene. There are two subclasses of gene mutations; one is called *point mutations* and the other *intragenic deletions*. Point mutations may involve the displacement of one nucleic acid base by another (base-pair substitution) and result in substitution of one amino acid for another in the final gene product, which will alter cellular function. Alternatively, they may involve insertion or deletion of a nucleotide or nucleotides within a polynucleotide sequence of a gene (frame-shift mutations). This leads to alteration in the nucleotide sequence, thus producing an incorrect gene product. An intragenic deletion occurs when a more extensive deletion happens within a gene, so that the informational material of that gene is essentially lost.

16.3 EFFECTS OF MUTATION

Mutations often induce deleterious effects on the individuals or populations involved. (While the effects of several individual mutagens are discussed further in this chapter, a general concept is addressed here.) One of the concerns over mutagenic agents is their relationship with cancer. As is widely recognized, the majority of human cancers appear to be related to environmental factors, and many mutagens have been shown to be carcinogens, or cancer-causing agents. However, in the long term, the ability of different environmental agents to cause mutations (and teratogenic effects) may create a greater burden on society than cancer does because of the increased incidence of genetic disease and birth defects.

The total impact of genetic disease on national health is unknown. Autosomal dominant disorders have been shown to occur in 8 of 10,000 births (Stryer 1988). A newspaper in British Columbia, Canada, reported that 9.4 individuals of every 100 live births suffer from genetic diseases or disabilities, and that 2.7 of every 100 live births have disorders of unknown etiology that may be partly genetic.

Serious consequences can result if a mutation occurs in such a way that, in a resultant protein, a hydrophilic amino acid is substituted for a hydrophobic residue or vice versa. A typical example is sickle cell anemia, a hereditary disease. This disease is the result of a biochemical lesion caused by substitution of glutamic acid (a hydrophilic amino acid) for valine (a hydrophobic amino acid) in a chain of approximately 140 amino acids in human hemoglobin. This seemingly minor change causes the production of abnormally shaped red blood cells that can no longer transport oxygen efficiently, leading to detrimental anemia.

Conversely, mutations may not necessarily produce deleterious effects on an organism. For instance, if mutations occur in such a way that only one amino acid along the backbone of a protein is incorrectly specified, the three-dimensional structure of the protein may not be greatly altered, allowing it to function properly. This is usually the case when a hydrophilic amino acid residue in a protein is replaced by another hydrophilic amino acid or a hydrophobic–hydrophobic replacement occurs. Occasionally, a mutation may occur that results in an enhanced ability of a cell or a species to survive. However, humans are highly developed organisms, so when a mutation does occur, the probability is that it will be a deleterious one.

16.4 INDUCTION OF MUTATION

Commonly found mutagens that are of most concern to humans include ultraviolet (UV) light, ionizing radiation, microtoxins, and organic and inorganic chemicals. Some common environmental mutagens and their sources are listed in Table 16.1.

16.4.1 UV Light

The region of the electromagnetic spectrum with wavelengths between 200 and 300 nm is of primary biological importance. The main reason for this is that DNA absorbs most strongly at 260 nm. It has been shown that mutations in microorganisms can be caused by irradiation of growth medium by UV light. Production of mutations by UV light, however, is strongly influenced by repair processes that reverse or remove induced photoproducts in DNA.

One of the most important ways in which the biological activity of DNA is altered by UV irradiation is thymine dimerization, a reaction in which two thymine molecules are fused together to form a dimer (Figure 16.3a). This dimerization may occur between adjacent thymine residues or between two thymine residues across the chains (interchain dimerization). Dimerization results in disruption of hydrogen

TABLE 16.1
Common Environmental Mutagens

Mutagen	Sources
UV light	Sunlight
Ionizing radiation	Cosmic rays, medical X-rays
Nitrosamines	Pyrolysis products of tryptophan, broiled meat, beer, and whisky
Benzo[a]pyrene	Cigarettes and wood smoke
Benzidine	Textile dyes, manufacture of paper and leather
Cr(VI), Hg	Metal alloys, mines
Hydrazine	Cigarettes and wood smoke
Malonaldehyde	Peroxidized polyunsaturated fatty acids
Vinyl chloride	Plastics
Aflatoxin B1	Fungi-contaminated grains and peanut

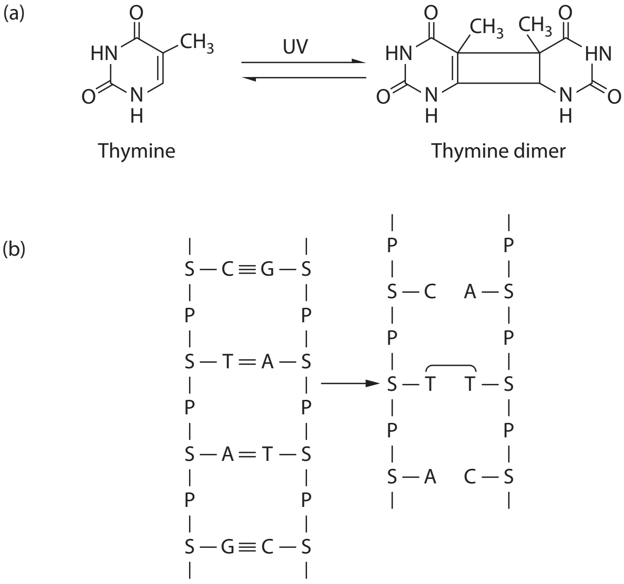


FIGURE 16.3 (a) UV radiation-initiated formation of a thymine dimer; (b) interchain dimerization disrupts hydrogen bonding between DNA bases.

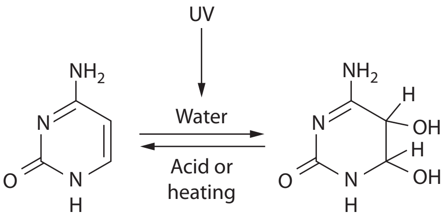


FIGURE 16.4 Hydration of cytosine.

bonding between the bases in the DNA molecule (Figure 16.3b). Chain break (P-S-P-S) is another possible result. UV irradiation can also cause hydration of cytosine (Figure 16.4), which may also result in hydrogen bond disruption. The effect of UV irradiation is not limited to DNA. Proteins and RNA outside the nucleus and other cellular components may also be affected.

16.4.2 IONIZING RADIATIONS

Examples of ionizing radiations include X-rays, γ-rays, α-particles, high-energy neutrons, and electrons. Ionizing radiation produces various kinds of DNA damage, such as altering DNA bases, or produces single- or double-stranded breaks in the phosphodiester chains of the DNA molecule, leading to fragmentation of the DNA. Such damages will consequently change the coding properties of DNA, resulting in induction of mutations (Breimer and Lindahl 1985).

TABLE 16.2
Mechanisms of Action of Several Mutagenic Agents

Chemical Action	Mechanism of Action
Alkylation	Addition of an alkyl group ($CH_3CH_2CH_2^-$, etc.) to a nucleotide
Arylation	Covalent bonding of an aryl group
Intercalation	Insertion of compound into DNA, altering the dimension or properties of the helix
Base analog incorporation	Base-pairing errors due to incorporation mispairing
Metaphase poisons	Interference with spindle formation and disruption of migration and segregation of chromosomes
Deamination	Removal of an amino group (NH_2) from adenine, cytosine, or guanine
Enzyme inhibition	Interference with biosynthesis of purines or pyrimidines and interference with repair

16.4.3 CHEMICAL MUTAGENS

More than 80,000 commercial chemicals are in use in the United States, and this number is increasing by 1,000 new compounds yearly (Ames 1979). There are also many environmental chemicals that are of concern. Some of these are derived from the commercial chemicals, while others are produced from anthropogenic sources. Anthropogenic sources include industrial processes involving combustion of fossil fuels; transportation; emissions from the open burning of scrap rubber tires; combustion of agricultural wastes (such as sugarcane, orchard prunings, and grain straws); municipal sewage sludges (Babish et al. 1983); herbicides such as sulfallate; and textile manufacturing. Mutagenic compounds have been classified into seven major categories based on their actions on DNA. These categories are (Graedel et al. 1986) as follows:

- alkylation
- arylation
- intercalation
- base analog incorporation
- metaphase poisons
- deamination
- enzyme inhibition

Table 16.2 summarizes the mechanisms involved in these categories. Some examples are shown in the following sections.

16.4.3.1 Alkylating Agents

Alkylating agents represent the largest group of mutagens. They may carry a single reactive group, the monofunctional alkylating agents, or two or more reactive alkyl groups and are thus called bi- or polyfunctional alkylating agents, respectively. These compounds can cause base alkylation, depurination, backbone breakage, or alkylation

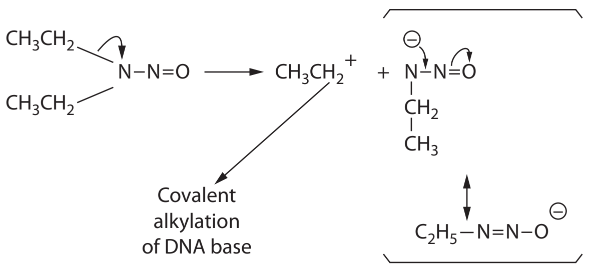
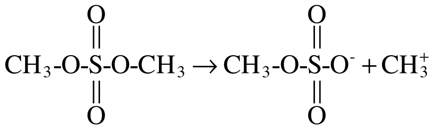


FIGURE 16.5 Diethylnitrosoamine is an alkylating agent.

of phosphate groups. For example, dimethyl sulfate may yield CH_3^+ (Reaction 16.1), which may attack the N–7 position of guanine (G) or N–3 position of adenine (A).



$$\mathrm{CH_3\text{-}O\text{-}\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}\text{-}O\text{-}CH_3 \rightarrow CH_3\text{-}O\text{-}\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}\text{-}O^- + CH_3^+} \tag{16.1}$$

Dimethyl sulfate

In addition, most nitroso compounds are highly mutagenic (and carcinogenic) because of their ability to form electrophilic species. Figure 16.5 gives an example showing how diethylnitrosamine, a nitroso compound, can act as an alkylating agent. In this case, diethylnitrosamine is converted into two species, one of which is carbonium $CH_3CH_2^+$ ion. This ion may seek such nucleophilic sites as –N– or –S– on informational macromolecules, resulting in the covalent alkylation of a DNA base. For example, N–2 and N–3 of guanine (G) are highly susceptible to electrophilic attack. An alkylated G may not base pair properly, or the information content of the molecule is altered in some way by the mutation. For instance, the alkylated G now pairs with T instead of with C, thus causing transitional-type mutations. It is also possible for the alkyl group of N–7 to labilize the β-glycoside bond, resulting in depurination and leading to transition or transversion.

Some chemical mutagens, such as HNO_2, can react directly with nitrogenous bases of DNA (Figure 16.6). Other mutagens have structures that are similar to that of one of the bases; these are called *base analogs*. It is possible for these base analogs

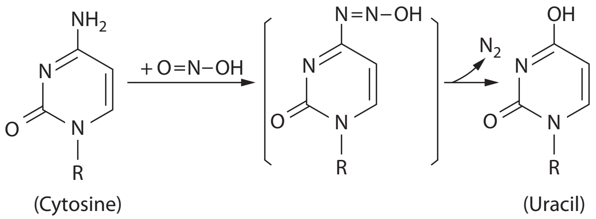


FIGURE 16.6 Formation of uracil through reaction of HNO_2 with cytosine.

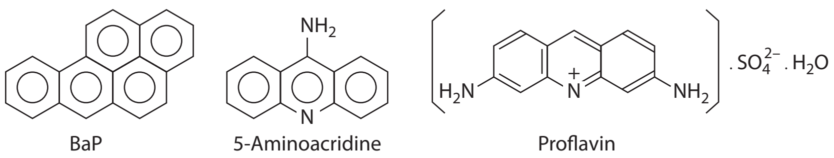


FIGURE 16.7 Examples of intercalating agents.

to be incorporated into a DNA molecule. For example, 5-bromouracil in its normal (keto) form hydrogen bonds with adenine (as would U or T) but in its enol form it base pairs with G.

16.4.3.2 Intercalating Agents

Many planar aromatic hydrocarbons are thought to be able to position themselves (intercalate) between the flat layers of H-bonded base pairs in the interior of the DNA double helix, forcing it to uncoil partially. Such compounds are often called *intercalating agents*. As a result of their action, errors occur in the transmission of the genetic code. Examples of such intercalating agents include BaP (benzo[a]pyrene), 5-aminoacridine, and proflavin (Figure 16.7).

16.4.3.3 Metals

A variety of metallic salts can induce cytotoxic effects, leading to denaturation of macromolecules. Certain metals are particularly important in this aspect because some of their ions can react with nucleic acids, initiating mutagenesis and carcinogenesis. As noted in Chapter 12, exposure to mercury (Hg) resulted in decreased DNA content in cells. Mercury also adversely affects chromosomes and mitosis, leading to mutagenesis.

The crucial factors in the toxic action of metals such as mercury may involve specific reactions with certain chemical groups in biomolecules or with certain sites in tissues or organelles. Examples are given in Chapter 12, showing the interaction of mercury and lead (Pb) with the –SH group in proteins. A specific example showing the interaction of lead with δ-aminolevulinic acid dehydratase (ALA-D) in heme biosynthesis is presented. As already noted, some toxic metals can compete with essential metals such as magnesium (Mg), calcium (Ca), or zinc (Zn). These metals are required as cofactors in a number of enzyme systems, or they may contribute to stabilizing the structure of biomolecules. Research has shown that different metallic ions react with different ligands (Jacobson and Turner 1980). Mg^{2+} and Ca^{2+} ions, for example, bind to phosphate groups on nucleotides and tend to stabilize the DNA double helix, whereas mercury and silver (Ag) bind to bases, lowering the stability of the helix.

Several studies have shown that chromium [Cr(VI)] compounds induce chromosome aberrations and mutations in cultured mammalian cells (Majone and Levi 1979; Tsuda and Kato 1977). Induction of DNA single-strand breaks and DNA-protein crosslinks by Cr(VI) compounds has also been reported (Sugiyama et al. 1991). Cr(VI) compounds can also inhibit the activity of such enzymes as glutathione reductase in cultured cells. After it enters the cell, Cr(VI) is reduced to Cr(III) through the intermediates Cr(V) and Cr(IV). This reduction process is accompanied by the formation of radical species such as active oxygen (Kawanishi et al. 1986), as well as glutathionyl radicals (Shi and Dalai 1989). These are considered to be responsible for the observed chromate-induced DNA damage. It has been shown that pretreatment with α-tocopherol (vitamin E) reduced the chromosomal aberrations caused by chromium. It is thought that because vitamin E is an efficient free-radical scavenger it may scavenge Cr(V) and free radicals (Sugiyama et al. 1991).

REVIEW QUESTIONS

1. Define the term *mutation*.
2. How are chromosomal aberrations different from gene mutations?
3. Match the item in the left column with an item in the right column:
    - (1) Inversion — (a) A portion of a chromosome is lost.
    - (2) Deletion — (b) The order of genes on a chromosome is reversed in one area.
    - (3) Translocation — (c) An additional copy of a portion of the chromosome is inserted.
    - (4) Duplication — (d) A broken portion of a chromosome attaches itself to a second chromosome
4. Which of the following is more deleterious to an animal or a person?
    - a. Substitution of a hydrophobic amino acid for another hydrophobic amino acid
    - b. Substitution of a hydrophilic amino acid for a hydrophobic amino acid
5. How does UV radiation affect DNA?
6. How do ionizing radiations affect DNA bases?
7. What is dimerization? Which environmental agent(s) can cause it?
8. Describe the way in which alkylation may induce mutation.
9. Give an example to explain the term *intercalation*.
10. How does mercury interact with the DNA helix?
11. Which is more toxic, Cr(III) or Cr(VI)?
12. Why is chromium mutagenic?
13. Explain why vitamin E appears to reduce the toxicity caused by Cr(VI).

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17 Environmental Cancer

17.1 INTRODUCTION

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. In the scientific or medical community, the term *malignant neoplasm* (tumor) is often used in place of cancer. Malignant tumors develop most commonly in major organs, such as the lungs, liver, stomach, intestines, skin, breasts, or pancreas, but they may also develop in lips, tongue, testes, or ovaries. Cancer may also develop in the blood cell-forming tissues of the bone marrow (the leukemias) and in the lymphatic system or bones.

In recent decades, there has been a growing concern about the possible effects of a large number of environmental toxicants on carcinogenesis. As noted in previous chapters, cancer incidence and mortality have increased dramatically over the past century. Researchers consider that there are two main reasons for the observed increase: the aging of the population and an increase in pollution from carcinogens present in and released into the environment through human activities. Studies showed that nearly 30% of the total mortality in many industrialized countries is attributed to cancer. In the United States, cancer remains the number two killer, accounting for nearly one-fourth of all deaths. Despite the recent decline in the mortality rate, the total number of cancer deaths continues to rise as the elderly population increases. For example, the death toll in the United States in 1980 was 416,509; in 1995, it was 538,455 (National Center for Health Statistics 1997), and it was estimated to be 569,490 in 2010, more than 1,500 people a day (American Cancer Society 2010).

One of the most common characteristics of the development of a neoplasm in an organism is the long period of time between the initial application of a carcinogen (cancer-causing agent) and the appearance of a neoplasm. The latency period varies with the type of carcinogen, its dosage, and certain characteristics of the target cells within the host. In humans, cancer may not be manifested until at least 10 years or longer after the initial exposure to a carcinogen occurs.

17.2 CAUSES OF CANCER

Cancer may be caused by many factors, and several factors, both inside and outside the body, contribute to development. Scientists usually divide those factors into two categories: those inside the body and those outside the body or environmental factors. Certain factors inside the body make some people more likely to develop cancer than others. For instance, some people may have inherited or may acquire the conditions, such as altered genes in body cells, abnormal hormone levels in the bloodstream, or a weakened immune system. Each of these factors may cause an individual to become more susceptible to cancer induction. They include diet, alcohol, smoking, reproductive and sexual behavior, occupational hazards, geographical

TABLE 17.1
Speculative Proportion of Cancer Deaths Attributed to Various Factors

Factor or Class of Factors	Percentage of All Cancer Deaths
Diet	35
Tobacco	30
Reproductive and sexual behavior	7
Occupational hazards	4
Geophysical factors	3
Alcohol	3
Pollution	2
Industrial products	1
Medicine and medical procedures	1
Infection	10?
Unknown	?

Source: Adapted from U.S. Department of Health and Human Services. *The surgeon general's report on nutrition and health.* U.S. Government Printing Office, Washington, DC, 1988, 177.

factors, and environmental agents. An estimate of the contribution of various agents or lifestyle to the cause of cancer is presented in Table 17.1. It is notable that diet and smoking account for approximately two-thirds of all cancers. Smoking is particularly implicated in lung and bladder cancers.

Although there are many theories concerning the causes of cancer, the fundamental principle underlying these theories is the alteration of the genetic material of the cell, the DNA. The various theories attempt to explain how this change is brought about. The DNA of a cancer cell is slightly different from that of a normal cell. This means that the sequence of the bases—adenine (A), guanine (G), thymine (T), and cytosine (C)—in a given strand of DNA is not the same as that of the bases in a normal cell. As mentioned in Chapter 16, these sequences dictate the sequences of the transcribed messenger RNA (mRNA), which in turn specifies the kinds of proteins to be synthesized in a cell. Alteration in the DNA base sequence in cancer cells leads to production of abnormal proteins. These new proteins influence the mechanisms of growth control in such a way that cell division continues indefinitely.

As mentioned, several types of DNA damage can occur. The most common types are (a) single- and double-strand breaks in the DNA backbone; (b) formation of cross-links between DNA bases and between DNA bases and proteins; and (c) chemical addition to the DNA bases. These alterations can result from exposure to radiation, and chemical, biological, and genetic factors (Table 17.2). For example, ionizing radiation such as X-rays and λ-rays can produce DNA single- and double-strand breaks and various forms of changes to bases. Ultraviolet (UV) light, which is a nonionizing radiation, can cause the production of dimers, where two DNA

TABLE 17.2
General Classification of Carcinogenic Agents

Class	Example
Radiation	Ultraviolet and ionizing radiations
Chemical	Polycyclic aromatic hydrocarbons, aromatic amines and halides, benzene, vinyl chloride, aflatoxin B1, urethane, asbestos, certain metals, diet, and tobacco smoke
Genetic	Viruses
Biological	Transgenesis by enhancer-promoter-oncogene constructs

bases are linked together. A variety of chemicals can cause DNA damage through base alterations. Alteration may be induced directly through formation of adducts or indirectly through intercalation formed by a chemical between two bases (Chapter 16). Many electrophilic chemicals can react with DNA, forming covalent additional products termed *adducts*. For example, alkylating agents can yield a reactive alkyl group that can react with base material, such as guanine, to produce an adduct.

17.3 THREE STAGES IN THE DEVELOPMENT OF CANCER

It is generally accepted that the pathway leading to carcinogenesis includes three stages: initiation, promotion, and progression (Figure 17.1) (U.S. Department of Health and Human Services [USDHHS] 1988). Initiation results from a simple mutation in one or more cellular genes that control key regulatory pathways of the cell. It requires cell division for the fixation of the process. Unlike promotion or progression, initiation is irreversible in a viable cell (Pitot and Dragan 2001). The efficiency of initiation is sensitive to xenobiotic and other chemical factors, and the stage can be altered by both endogenous and exogenous factors. For example, a variety of chemicals in different tissues can inhibit the metabolism of a certain chemical to an ultimate carcinogen, thereby blocking the initiation process. Initiators can also produce transformed cells that may persist for the life span of an individual without producing cancer. In such cases, the damaged gene in the transformed cells remains recessive because the gene is not expressing an abnormal protein.

Promotion results from the selective functional enhancement of signal transduction pathways induced in the initiated cell and its progeny by the continuous exposure to the promoting agent (Pitot and Dragan 2001). Promotion involves gene activation, leading to the synthesis of the abnormal protein. Rapid cell division occurs, and this is followed by interruption of the normal function or health of the organism. Promotion then leads to the expression of genetic changes as malignancy, which involves loss of control over cellular proliferation. Examples of promoting agents include saccharin, butylated hydroxytoluene, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (see Chapter 13), and androgens and estrogens. In contrast to initiation, promotion is reversible. Therefore, if the promoting agent is withdrawn well before tumors are manifested, the appearance of tumors can be delayed or prevented. Furthermore, promotion may be continually modulated by various environmental factors, such as frequency with which the promoting agent is administered, age and sex of the subject, hormonal

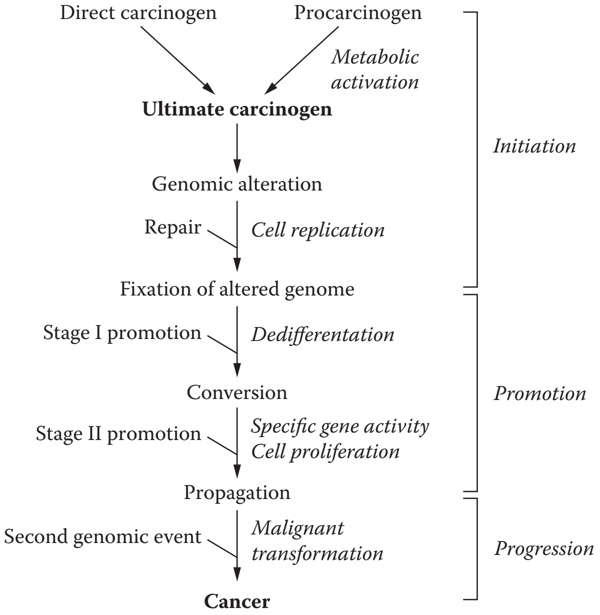
Direct carcinogen
Procarcinogen
Metabolic activation
Ultimate carcinogen
Genomic alteration
Repair
Cell replication
Fixation of altered genome
Stage I promotion
Dedifferentation
Conversion
Stage II promotion
Specific gene activity
Cell proliferation
Propagation
Second genomic event
Malignant transformation
Cancer
Initiation
Promotion
Progression

FIGURE 17.1 Three stages of carcinogenesis. (Adapted from U.S. Department of Health and Human Services. *The surgeon general's report on nutrition and health.* U.S. Government Printing Office, Washington, DC, 1988, 177.)

balance, and dietary factors. Researchers have shown that many promoting agents exert their effects on the cell through mediation of receptor mechanisms (Pitot and Dragan 1991). Certain chemicals act as initiators as well as promoters. An example is benzo[a]pyrene (BaP; Chapter 16). In small doses, this substance can initiate genetic damage; in higher or repeated doses, it enhances promotion.

Promoting agents involved in the onset of promotion do not cause cancer by themselves; rather, they only have a certain impact on an initiated cell. The process of promotion is gradual, and some of the earlier steps are reversible. In the promotion stage, abnormal proliferation of the affected cell occurs, presumably by the presence of high concentrations of growth factors or modified cell-surface receptors. If the damage to the gene is not drastic, most of the normal components of the cell will be produced and will be responsive to normal growth-inhibiting factors. Experiments with animals suggest that the time lapse between initiation and promotion is not critical. During the late stage of promotion, however, cumulative genetic changes occur, leading to irreversible neoplastic transformation. Progression results from continuing evolution of an unstable karyotype. This stage usually develops from cells in the stage of promotion, but in certain conditions, it may develop directly from normal cells. The critical molecular characteristic of this stage is karyotypic instability, and morphologically discernible changes in cellular or genomic structure occur (Pitot and Dragan 2001). Furthermore, benign or malignant tumors may be formed in this stage. The growth of altered cells is sensitive to environmental factors during the early phase of progression.

17.4 METASTASIS

Metastasis is the spread of malignant cells from the primary site to other parts of the body. This is the most fearsome aspect of cancer, and it occurs in the late stage of the disease. It is characterized by invasive activity and the appearance of a variety of cancer cell types. Some of the cells that have the inherent ability to detach from the primary site eventually travel through blood vessels or lymph to start a secondary tumor at another site. Metastasis that occurs in cancer patients is the primary cause for the failure of their treatment. The extent of the dissemination of the malignant cells is determined by the physiological condition of the host. During metastasis, continuous changes occur in the tumor, and the function and behavior of the tumor cells in the late stage are quite different from those in the early stage. Most frequently, the location of metastasis is in the organ or organs that are served by blood vessels from the original cancer site. Notably, growth and survival of a tumor require nourishment, which is provided by new blood vessels near the tumor site.

17.5 CLASSIFICATION OF CARCINOGENS

As noted earlier, carcinogens are cancer-causing agents. According to the list prepared by the U.S. National Toxicology Program (NTP), carcinogens are divided into two groups: Part A and Part B (see Appendix 2). Part A includes those agents that are "known to be human carcinogens," whereas Part B refers to those that are "reasonably anticipated to be a human carcinogen." Examples of carcinogens belonging to Part A include aflatoxins, inorganic arsenic compounds, benzene, asbestos, beryllium, coal tars, dioxin, diethylstilbestrol, tobacco smoke, steroidal estrogens, nickel compounds, radon, vinyl bromide, vinyl chloride, and UV radiation. Nearly 200 agents are included in the table (Appendix 2).

According to a long-awaited Environmental Protection Agency (EPA) draft report released on May21, 2010, dioxins are carcinogens and can cause other health effects. The draft says there is no threshold below which dioxins pose no cancer risk. To protect against noncancerous effects, the draft, for the first time, sets a safe daily dose of 7×10^{-10} mg per kg of body weight per day for TCDD, the most toxic form of dioxin. Once finalized, the reassessment will influence many regulatory decisions affecting the group of compounds collectively called dioxins. The draft report updates EPA's first official risk estimates for dioxins set in 1984 and based solely on cancer concerns. The EPA has been reassessing the risks of dioxins since 1991 (Pitot and Dragan 2001). A report in *Chemical & Engineering News* indicated that the director of the EPA instructed the agency to finalize its dioxin reassessment by the end of 2010 (USEPA 2010).

As noted, the basic changes in DNA that can lead to cancer (i.e., mutation) can be caused by many agents. These agents are generally divided into four categories: radiation, chemical, biological, and genetic (Table 17.2) (USDHHS 1988). Although mutation does not necessarily result in cancer, cancer occurs if the proteins that are produced following mutation affect cellular growth-control mechanisms. The following section discusses in some detail the agents that can cause DNA damage, with an emphasis placed on radiation and chemical agents.

17.5.1 Radiation

The process involved in radiation-induced DNA damage is complex and has received much attention for many years. As mentioned, ionizing radiation produces a wide variety of DNA lesions, such as base modifications, strand breaks, and DNA-protein cross-links (Pitot 1986). It was mentioned in Chapter 16 that absorption of short-wave UV radiation by DNA causes breakage in the double strand, the opening of the rings of DNA bases, and the formation of thymine dimers (two molecules of thymine linked together).

UV radiation is the main cause of skin cancer. Increased UV radiation exposure—much of it caused by sunbathing or tanning under a UV lamp—is the main contributing factor to the rising incidence or skin cancer worldwide. UV radiation induces free-radical formation, especially reactive oxygen radicals. Of the three types of UV radiation (UV-A, UV-B, and UV-C), UV-B is most harmful. UV-B has a wavelength of 280 to 320 nm and is attenuated by the earth's ozone layer. Several other factors modulate the amount of UV radiation to which people are exposed. These include time of day, season, humidity, and distance from the equator. Skin cancer risk is also affected by skin type; fair skin that freckles or bruises easily is more at risk than very darkly pigmented skin. People who live in sunny climates and have red or blond hair and blue or light-colored eyes are at especially high risk.

Among the photochemical reactions that take place when UV-B penetrates the skin is mutation of the DNA in skin cells. Humans have repair enzymes that can correct this damage, but mutations accumulate as the individual ages. An individual's lifestyle may also cause the repair system to eventually become overtaxed, resulting in skin cancer. Most researchers stress that the damage begins accumulating early in childhood; by young adulthood, about 50% of lifetime sunlight exposure may have already accumulated.

17.5.2 Chemical Carcinogens

The association between exposure to chemicals with cancer incidence was first reported in 1775 by the English physician Percivall Pott, following the observation of scrotal cancer in chimney sweeps (Cole and Goldman 1975). With an increase in European industrial development during the nineteenth century, high skin cancer rates were observed among workers in the shale oil and coal tar industries. In 1915, a group of Japanese scientists conducted experiments in which the scientists painted rabbits with coal tar and induced tumors. This led to the knowledge that the compounds contained in the coal tar could produce cancer in animals. Several groups of organic compounds have now been recognized as carcinogenic to experimental animals. These include benzene, polycyclic aromatic hydrocarbons (PAHs), aromatic amines, aminoazo dyes, nitroso compounds, and vinyl chloride (Pitot 1986).

Many chemical agents that may be found in foods are also known to cause cancer. For example, aflatoxin B1, which causes liver cancer in several species of test animals, is produced by *Aspergillus flavus,* found in contaminated peanut or cottonseed meal. There are also naturally produced substances that are carcinogenic.

A number of inorganic substances have also been shown to induce cancer. These include some salts of arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr) (VI), nickel (Ni), and beryllium (Be). Cadmium is also known to be embryotoxic. Since 1994, cadmium has been accepted by the International Agency for Research on Cancer (IARC) as a human carcinogen, based primarily on its association with pulmonary tumors. It should be pointed out that some of the metals listed are essential nutrients for humans and animals. Trivalent chromium Cr(III) is one of these metals. As part of the "glucose tolerance factor," chromium plays an important role in maintaining normal glucose metabolism in mammals.

As mentioned in Chapter 2, an unusual linkage between coal and cancer in China's Yunnan Province was found (Bhanoo 2010). Nonsmoking women in an area in the province die of lung cancer at a rate 20 times that of their counterparts in other regions of the country and anywhere else in the world. Subsequently, coal samples were analyzed by scientists from the United States. They found that quartz, of which silicon (Si) is the primary component, made up 13.5% of the coal samples taken from Xuanwei County in Yunnan Province. The high levels of quartz in the coal samples are exceptional in that, in normal coal samples, quartz and other minerals are found only in trace amounts. The high cancer rates in Xuanwei have attracted the attention of scientists for decades.

A group of scientists is saying now that a possible explanation is the burning of coal formed during volcanic eruptions hundreds of millions of years ago. It was found that the coal in that part of China contains high concentrations of silica, a suspected carcinogen. Like others in rural China, the families of Xuanwei County use coal for heat and for cooking. As the coal burns, particles of silicon are released with the vapor and inhaled. Women, who do the cooking, face the greatest exposure. An epidemiologist at the National Cancer Institute in Rockville, Maryland, is completing two studies involving hundreds of women and families in Xuanwei County. While her team is confident that coal burning is causing the high rates of cancer, they are not certain it is due to silica.

17.6 METABOLISM OF CHEMICAL CARCINOGENS

As shown in Figure 17.1, chemical carcinogens are normally divided into two broad classes: direct carcinogens and procarcinogens. Direct carcinogens are usually electrophiles, such as H^+, C^+, and N^+, and can readily react with nucleophiles, such as proteins and nucleic acids. The main sites in these molecules where such reactions can occur are S, =N–, –C–OH, or –P–OH. Examples of cellular nucleophiles include some amino acids, such as methionine, cysteine, histidine, tryptophan, and tyrosine and nucleic acid bases such as adenine (N-l, N-3) and guanine (C-8, N-7, O-6).

Procarcinogens are those agents that require biologic activation before becoming ultimate carcinogens. Compared with direct carcinogens, procarcinogens are stable enough so that many people may be environmentally or occupationally exposed to them. It is possible for people to ingest or absorb some procarcinogens, which then go through enzymatic conversion in the liver, lungs, or other organs to their activated metabolites.

It is thought that most, and probably all, chemical carcinogens are converted by metabolism into electrophilic reactants that exert their biological effects by covalent

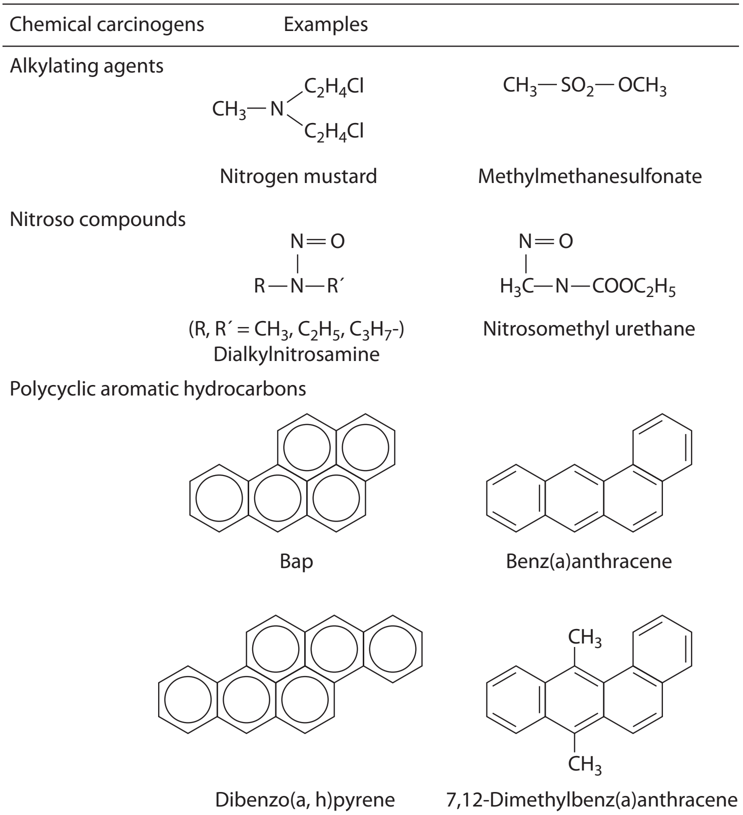
Chemical carcinogens
Examples
Alkylating agents
CH3—N(C2H4Cl)(C2H4Cl)
Nitrogen mustard
CH3—SO2—OCH3
Methylmethanesulfonate
Nitroso compounds
N=O
R—N—R′
(R, R′ = CH3, C2H5, C3H7-)
Dialkylnitrosamine
N=O
H3C—N—COOC2H5
Nitrosomethyl urethane
Polycyclic aromatic hydrocarbons
Bap
Benz(a)anthracene
Dibenzo(a, h)pyrene
CH3
CH3
7,12-Dimethylbenz(a)anthracene

FIGURE 17.2 Examples of chemical carcinogens.

interaction with DNA. Some examples of these reactants are shown in Figure 17.2. Several of these chemicals are discussed in more detail in the following sections.

17.6.1 Free Radicals

Reactive oxygen species, such as hydroxyl radicals (OH·), are produced during the enzymatic and chemical reactions of molecular oxygen in cells. Hydroxyl radicals are also produced when cells are exposed to ionizing radiation, tumor promoters, and chemical carcinogens. As mentioned, reactive oxygen species can cause various lesions in DNA, by inducing damage to nucleic acids and altering their structures and function. Oxygen-induced lesions of nucleic acids include strand breaks (Floyd 1990) and base modification products. Alternatively, the OH· free radical, formed through the reaction between superoxide free radical ($O_2\cdot^-$) and H_2O_2 (Equation 17.1), is unique and can induce breaks in the phosphodiester bonds. Both single- and double-strand breaks can occur. In addition, the free radical can abstract hydrogen atoms from the DNA helix (Cardenas 1989).

$$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + HO^- + HO\cdot \tag{17.1}$$

17.6.2 DDT

DDT is one of several pesticides that have been added to the long list of cancer-causing agents present in the environment. According to a report by the National Cancer Institute, women with high exposure to DDT may have a greater risk of developing breast cancer. Researchers at Mt. Sinai Hospital in New York City have found that women with blood levels of DDE [ethylene 1,1-dichloro- 2,2-bis(p-chlorophenyl)] (see Chapter 13) at 19 ng/mL have four times the risk of breast cancer than women with DDE levels of 2 ng/mL.

It is suggested that DDE may cause breast cancer in two ways: It may induce cytochrome P450 enzymes, thereby altering the metabolism of toxicants, or it may act as an estrogen mimic that may disrupt the endocrine system through interaction with estrogen receptors (see Chapter 15).

17.6.3 FORMALDEHYDE

In a draft toxicological assessment published in May 2010 by the EPA, formaldehyde is carcinogenic when inhaled by humans (Wajert 2010). According to the draft, "There is sufficient evidence of a cancer relationship between formaldehyde exposure and cancer of the upper respiratory tracts, with the strongest evidence for nasopharyngeal and sino-nasal cancers." There is also sufficient evidence of a causal association between formaldehyde exposure and lymphohematopoietic cancers, with the strongest evidence of Hodgkin's lymphoma and leukemia, particularly myeloid leukemia, according to the EPA.

17.6.4 VINYL CHLORIDE

Vinyl chloride, the common name for monochloroethene (CH_2=CHCl), is one of the most widely manufactured organic chemicals in the United States. It is a gas at ambient remperature, with a boiling point of 14°C, and exhibits a low solubility in water. While the vinyl chloride monomer itself is rarely used, it is polymerized with itself and other organic compounds to form many products, thus making it an important chemical to industry and consumers.

Among the many polymers that are derived from vinyl chloride, polyvinyl chloride (PVC) is the most common. PVC, as a solid material, is extremely adaptable and cost effective, and is used in numerous construction materials, home furnishings, packaging materials, automobile products, and so on. Some examples of the products made of PVC are water pipes, raincoats, credit cards, wire coatings, and food packaging.

PVC production involves three stages: synthesis of vinyl chloride monomer from petrochemicals and chlorine, polymerization of vinyl chloride into PVC resin, and PVC fabrication. Environmental contamination occurs from these processes, although the extent of it varies with each stage. The contamination includes emission of vinyl chloride into the atmosphere and surface and groundwater contamination resulting from sludge and wastewater discharge.

Vinyl chloride has been shown to be both mutagenic and carcinogenic. It is classified as a Part A carcinogen because sufficient evidence exists that the compound is

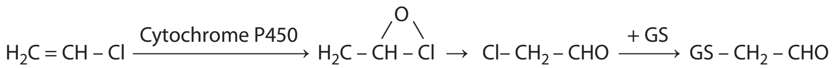

FIGURE 17.3 Metabolism of vinyl chloride by the cytochrome P450 system.

carcinogenic to humans. This is very important because only about 40 chemicals or chemical mixtures are classified as such (Van Duureen 1989). Vinyl chloride causes liver cancer in both humans and laboratory animals. However, laboratory experiments with mice showed induction of not only liver cancer but also cancers of bone, skin, lung, brain, nephron, and mammary tissues (Van Duureen 1989; Moss 1985). In humans, vinyl chloride exposure may occur occupationally and nonoccupationally.

Vinyl chloride is metabolized by the hepatic cytochrome P450 enzymes to the carcinogenic epoxide form. Studies showed that this metabolite is an ultimate carcinogen. It reacts with DNA, causing it to change its function. In the liver, the active epoxide may be further converted to chloroethane aldehyde. A molecule of glutathione can conjugate the aldehyde, and the resultant conjugate may then be excreted (Figure 17.3).

17.6.5 Alkylating Agents

Alkylating agents are those chemicals that can react with DNA to produce alkylated DNA adducts (Chapter 16). Several groups of organic compounds can be metabolized to alkylating agents. An example is N-nitroso compounds, which consist of nitrosamines and nitrosamides. Nitroso compounds are found in various types of food, particularly meat and meat products (e.g., fried and cured meat products) and cheese. Small amounts of the compounds have been shown to occur in beer, and tobacco smoke also contains varying amounts. Industrial exposure to N-nitrosamines accounts for another environmental source. Occupation or industrial activities that may potentially lead to the exposure include metal cutting and rolling; leather tanning, rubber manufacture, hydraulic fluids handling, and producing or using amines in the chemical industry. In these activities, exposure is mostly through air and skin (Preussmann et al. 1979).

The importance of nitrosamines as environmental carcinogens was first postulated in 1962. Subsequent studies demonstrated the endogenous formation of such compounds from precursor amines and nitrite in vivo. The endogenous formation of N-nitroso compounds from precursor amines and nitrosating agents, particularly nitrite, is unique among the various chemical carcinogens. Nitrosatable amine precursors, such as secondary and tertiary amines, are natural constituents of food or contaminants of food, such as some pesticides that can be nitrosated. Nitrite is the most important nitrosating agent and is present in some food products. However, nitrite can also be formed from nitrate in saliva and possibly in the intestines. The pathway leading to the formation of an alkylating agent from dimethylamine is presented in Figure 17.4. The first step is nitrozation, in which dimethylamine reacts with nitrite to form dimethylnitrosamine, a nitroso compound. Metabolism of dimethylnitrosamine leads to the formation of a CH_3^+ radical, which can react with DNA, forming methylated DNA.

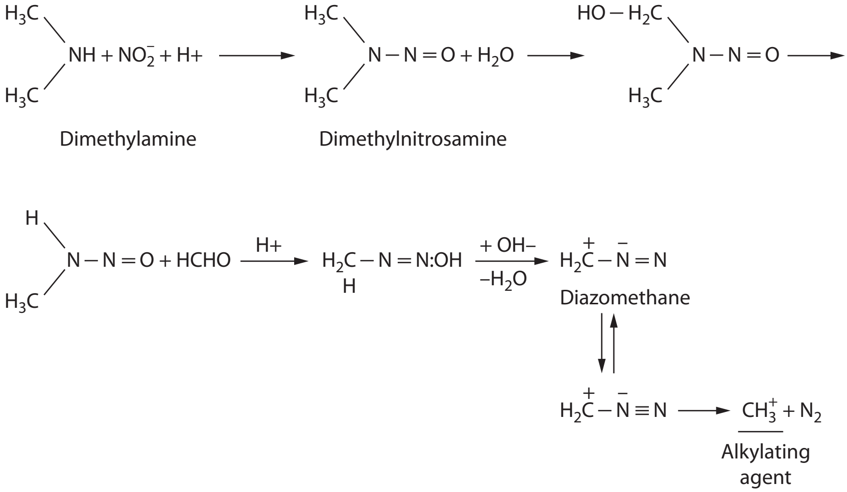

FIGURE 17.4 Activation mechanism of dimethylamine.

17.6.6 TRICHLOROETHYLENE (TCE)

The National Academy of Sciences has reported that a lot more is known about the cancer risks and other health hazards from exposure to trichloroethylene (TCE) than there was in 2005 when the EPA took steps to regulate it more strictly. TCE, which is also widely used to remove grease from metal parts in airplanes and to clean fuel lines at missile sites, is known to cause cancer in some laboratory animals. The EPA was blocked from elevating its assessment of the chemicals' risks in people by the Defense Department, Energy Department, and NASA, all of which have sites polluted with the chemical. Its 379-page report recommended that the EPA revise its assessment of TCE's risks using "currently available data"—so no more time is wasted. That is a step that could lead to stricter regulations. The EPA currently requires limiting TCE to no more than 5 ppb in drinking water.

17.6.7 POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds composed of two or more fused aromatic rings. They are emitted into the environment through both natural and anthropogenic combustion processes. The two main sources of natural PAH productions are volcanic eruptions and forest fires. Anthropogenic sources include fossil fuel combustion by automobiles and other transportation systems, petroleum refining processes, coking plants, asphalt production, industrial facilities that use fossil fuels, effluent disposal, oil spills, and refuse burning. PAHs are therefore widely distributed in all parts of the environment: air, soil, water, and sediments. They are of major concern because they represent a potential human health hazard through contamination of food and drinking water supplies. Estimated carcinogenic PAH concentrations in various environmental media are presented in Table 17.3 (Menzie et al. 1992).

TABLE 17.3
Estimated PAH Contents of Various Environmental Media

Environmental Medium	PAH Content
Outdoor air	2.6–13.0 ng/m^3
Indoor air	1.5–13.0 ng/m^3
Surface water	8.0 ng/L
Groundwater	1.2 ng/L
Drinking water	2.8 ng/L
Rural soil	0.07 mg/kg dry weight
Urban soil	1.10 mg/kg
Road dust	137 mg/kg
Charcoal broiled or smoked beef	35 μg/kg
Pork	26 μg/kg
Poultry	12 μg/kg
Fish/shellfish	0.10 μg/kg
Smoked fish/shellfish	36 μg/kg
Green leafy vegetables	46 μg/kg
Grains	9 μg/kg
Fruits	2.4 μg/kg
Fluid milk	0.09 μg/kg
Fats and oils	66 μg/kg
Cheese	1.70 μg/kg
Alcoholic beverages	0.08 μg/kg

17.6.7.1 Benzo(a)pyrene

Among the many PAHs, benzo(a)pyrene (BaP) (Figure 17.5) is probably the most widely known. In 1775, the British surgeon Percivall Pott first reported the relationship between the incidence of cancer of the scrotum among chimney sweeps and exposure to soot. Since then, many researchers repeatedly showed the potent carcinogenic effect of BaP. Sources of BaP emission include burning of coal and refuse, residential furnaces, coke production, vehicle disposal (open burning), wood burning, and forest and agricultural refuse burning. BaP is found in most commercial motor oil, asphalt roofing, and other construction materials, and tobacco smoke (Table 17.4). Like most PAHs, BaP is ubiquitous in the environment; it occurs in air, water, soil, and food. According to Menzie et al. (1992), the BaP concentrations in 58 prepared meals averaged 0.15 μg/kg, with a range of 0.005 to 1.17 μg/kg.

BaP can cause several forms of cancer, particularly cancers in the lung, intestine, kidney, and liver. It has been reported that the ultimate carcinogenic form of BaP is benzo(a)-7,8-diol-9,10-epoxide (Weinstein et al. 1976). This active form of BaP is formed through cytochrome P450-dependent activation followed by several enzymatic steps. The resultant BaP metabolite then forms an adduct with the base guanine in DNA, altering its function. Figure 17.5 summarizes the activation process.

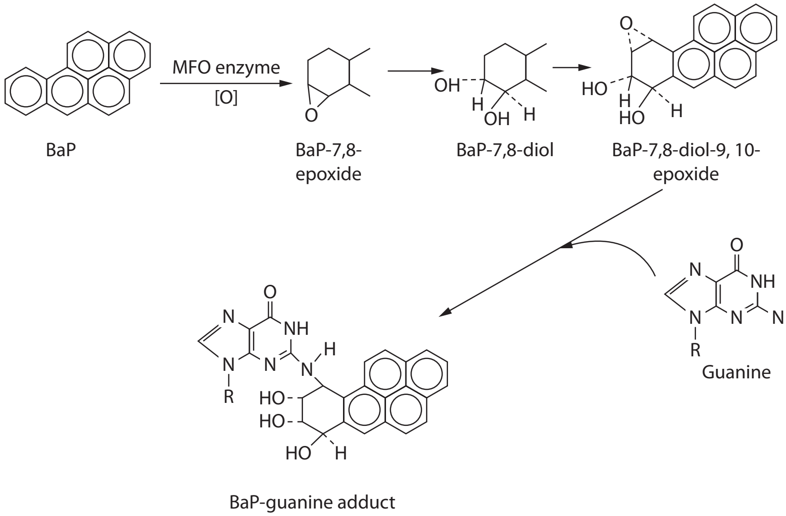

FIGURE 17.5 Formation of benzo(a)pyrene-guanine adduct.

TABLE 17.4
Chemicals, Groups of Chemicals, Complex Mixtures, and Industrial Processes Causally Associated with Cancer in Humans

4-Aminobiphenyl

Analgesic mixtures containing phenacetin
Arsenic and certain arsenic compounds
Asbestos
Auramine manufacture
Azathioprine
Benzene
Benzidine
Betel quid with tobacco
Bis(chloromethyl) ether and technical-grade chloromethyl methyl ether
Boot and shoe manufacture and repair
1,4-Butanediol dimethylsulfonate
Certain combined chemotherapy products
Chlorambucil
Chromium and certain chromium compounds
Coal gasification (older processes)
Coal tar
Coke production (certain exposures)
Coal tar pitch

Conjugated Estrogens

Cyclophosphamide
Diethylstilbestrol
Furniture manufacture (wood dusts)
Isopropyl alcohol
Melphalan
Methoxsalen with UV A therapy
Mineral oils (some)
Mustard gas
Underground hematite mining (with exposures to radon)
N,N-Bis(2-chloroethyl)-2-naphthylamnie
2-Naphthylamine
Nickel refining
Rubber
Shale oils
Smokeless tobacco
Soots
Tobacco smoke
Treosulfan industry (certain occupations)

Source: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Supplement No. 4 (Updated). (IARC: International Agency for Research on Cancer.)

17.6.7.2 Halogenated Aromatic Hydrocarbons

As discussed in Chapter 13, halogenated aromatic hydrocarbons, including polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs), are toxicants of much public and scientific concern because of their widespread distribution in the environment and the potential for human exposure from numerous sources. As previously noted, examples of the sources include combustion processes, contaminated pesticides, industrial accidents, or uncontrolled environmental discharge. Humans may be potentially exposed to these toxicants by such routes as the inhalation of vapors, aerosols, or respirable particles; the ingestion of contaminated milk, meat, fish, water, and vegetation; and the dermal absorption of contaminants in soil or from pesticides.

PCDDs and some other halogenated aromatic hydrocarbons have been shown to be carcinogenic in rats and mice (NTP 1980, 1982). Hepatocellular carcinomas were produced in both species following dioxin exposure, but only female rats developed squamous cell carcinomas of the lung, nasal turbinates, and hard palate (NTP 1982). Ingestion of 0.1 μg/kg per day in the diet resulted in an increased incidence of these tumors. In addition to hepatocellular carcinomas, follicular cell adenomas of the thyroid were significantly increased in male rats and female mice (NTP 1982). Other studies also reported a dose-dependent increase in tumors of estrogen-sensitive organs, including breast, uterus, and pituitary (Kochiba and Schwetz 1982). This suggests that PCDDs may be acting through a hormone-like mechanism. Most evidence suggests that the carcinogenic activity of PCDDs results from a receptor-mediated, nongenotoxic promotional mechanism (Shu et al. 1987; Kimbrough et al. 1975).

PCBs are carcinogenic in rodents, producing hepatocellular carcinoma in rats and hepatomas in mice (Kimbrough et al. 1975). The production of liver tumors in these species is related to the degree of chlorination of the PCB mixture.

The halogenated aromatic hydrocarbons are believed to produce their toxic effects through a common mechanism of action (Poland and Knutson 1982). These compounds are able to induce hepatic cytochrome P450 microsomal enzyme systems, especially aryl hydrocarbon hydroxylase (AHH) activity. It is generally accepted that the toxic activity of these chemicals is related to their interaction with a cellular receptor, the *Ah* receptor, which regulates the synthesis of a number of cellular proteins. As discussed in Chapters 13 and 14, the receptor is an intracellular protein that binds to these biphenolic compounds based on stereo-specific characteristics. Although the exact mechanism involved in the toxicity is not known, it is thought that the toxicity results from interference with the control of structural genes for several proteins through the binding of the *Ah* receptor (see Figure 13.12).

17.7 RESPIRATORY CANCER DEATH RATES

As noted in Chapter 2, following the Industrial Revolution, cancer death rates in the United States increased steadily until about the middle of the 1990s, when they began to diminish (Figure 2.4). The increases were particularly pronounced for respiratory cancer death rates. For instance, by use of the total cancer death rate of 1950 as a basis of comparison, the increase for total cancer death rate was 20%,

TABLE 17.5
Cancer Death Rates in United States between 1950 and 2006[a]

	Age-Adjusted Death Rates per 100,000 Population, by Year						
	1950	1960	1970	1980	1990	2000	2006
Deaths from all causes	1,410	1,311	1,193	1,012	909	849	764
Total cancer deaths	194	193	196	204	211	197	171
%	13.7	14.7	16.4	20.1	23.2	23.2	22.3
Percentage compared to 1950 rate	100	107	120	146	169	169	162
Deaths from respiratory system cancer	15	24.1	37.1	49.9	59.3	56.1	51.5
%	1	1.8	3.1	4.9	6.5	6.6	6.7
Percentage compared to 1950 rate	100	180	310	490	650	660	670

[a] Data from U.S. Department of Health and Human Services: *Health, United States, 2009*. U.S. Government Printing Office, Washington, DC.

46%, and 69% for 1970, 1980, and 1990, respectively. However, the death rate from respiratory system cancer increased 310%, 490%, and 650% for 1970, 1980, and 1990, respectively (Table 17.5).

Many researchers consider the observed increases to be the result of environmental pollution. Similarly, the dramatic decreases in the death rates shown in the last several decades were attributed to improved pollution control measures and practices seen in the country.

17.8 DNA REPAIR

17.8.1 DNA Damage

In human cells, both normal metabolic activities and environmental factors can cause DNA damage, resulting in as many as 1 million individual molecular lesions per cell per day (Lodish et al. 2004). Environmental factors include different chemical and physical agents, such as UV light, ionizing radiation, and a variety of chemicals. These factors can cause DNA bases to be altered or lost, the breakage of the phosphodiester bonds in the backbone, or the induction of covalent cross-linkages of the strands. It is important to note that much of the damage sustained by DNA can be repaired because genetic information is stored in both strands of the double helix; thus, information lost by one strand can be retrieved from the other (Stryer 1988).

17.8.2 Sources of Damage

DNA damage can be divided into two main types: endogenous damage and exogenous damage. Endogenous damage can occur as a result of attack by reactive oxygen species produced from normal metabolic by-products, especially the process of oxidative deamination. Exogenous damage, on the other hand, can be caused by a variety of external agents, such as UV (200–300 nm) radiation from the sun; other radiation frequencies, including X-rays and λ-rays; hydrolysis or thermal disruption; human-made mutagenic chemicals, especially aromatic compounds that act as DNA intercalating agents; cancer chemotherapy and radiotherapy; and viruses (Roulston et al. 1999).

17.8.3 TYPES OF DAMAGE

Five types of damage to DNA occur as a result of endogenous cellular processes:

- Oxidation of bases and generation of DNA strand interruptions from reactive oxygen species
- Alkylation of bases (usually methylation), such as 7-methylguanine and 1-methyladenine
- Hydrolysis of bases, such as deamination, and depurination
- "Bulky adduct formation," such as BaP diol epoxide-d-guanine adduct
- Mismatch of bases, due to errors in DNA replication, in which the wrong DNA base is stitched into place in a newly forming DNA strand or a DNA base is skipped over or mistakenly inserted

Examples of damage caused by exogenous agents include

- UV-B light causes cross-linking between adjacent thymine and cytosine bases, creating pyrimidine dimers. This is called *direct* DNA damage.
- UV-A light creates mostly free radicals. The damage caused by free radicals is called *indirect* DNA damage.
- Ionizing radiation such as that created by radioactive decay or in cosmic rays causes breaks in DNA strands. Low-level ionizing radiation may induce irreparable DNA damage, leading to premature aging and cancer (Acharya 1976).
- Thermal disruption at elevated temperature increases the rate of depurination (loss of purine bases from the DNA backbone) and single-strand breaks.
- Industrial chemicals such as vinyl chloride and H_2O_2 and environmental chemicals such as polycyclic hydrocarbons found in smoke, soot, and tar create a large variety of DNA adducts.

UV damage, alkylation or methylation, X-ray damage, and oxidative damage are examples of induced damage. Spontaneous damage can include the loss of a base, deamination, sugar ring puckering, and tautomeric shift (http://www.ncbi.nlm.nih.gov/books/bv.fcgi?).

17.8.4 DNA REPAIR

17.8.4.1 Introduction

Cells cannot function if DNA damage corrupts the integrity and accessibility of essential information in the genome. But, they remain superficially functional when so-called nonessential genes are missing or damaged. Depending on the type of damage inflicted on the double-helical structure of DNA, a variety of repair strategies has evolved to restore lost information. Damage to DNA alters the spatial configuration of the helix, and such alterations can be detected by the cell. Once damage is localized, specific DNA repair molecules bind at or near the site of damage, inducing other molecules to bind and form a complex that enables the actual repair to take place.

17.8.4.2 Direct Reversal

DNA repair generally refers to a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode its genome. Cells are known to eliminate three types of damage to their DNA by chemically reversing it. These mechanisms do not require a template since the types of damage they counteract can only occur in one of the four bases. Such direct reversal mechanisms are specific to the type of damage incurred and do not involve breakage of the phosphodiester backbone. The formation of pyrimidine dimers on irradiation with UV light results in an abnormal covalent bond between adjacent pyrimidine bases. The photoreactivation process directly reverses this damage by the action of an enzyme called photolyase, whose activation is dependent on the energy absorbed from blue/UV light (300- to 500-nm wavelength) to promote catalysis (Sancar 2003). Another type of damage, methylation of guanine bases is directly reversed by the protein methyl guanine methyl transferase. The third type of DNA damage reversed by cells is certain methylation of the bases cytosine and adenine.

17.8.4.3 Single-Strand Damage

When only one of the two strands of a double helix has a defect, the other strand can be used as a template to guide the correction of the damaged strand. A number of excision repair mechanisms exist that remove the damaged nucleotide and replace it with an undamaged nucleotide complementary to that found in the undamaged DNA strand. The mechanisms include

1. Base excision repair (BER): Repairs damage to a single base caused by oxidation, alkylation, hydrolysis, or deamination. The damaged base is removed by a DNA glycosylase. (The “missing tooth” is then recognized by an enzyme called AP endonuclease, which cuts the phosphodiester bond. The missing part is then resynthesized by a DNA polymerase, and a DNA ligase performs the final nick-sealing step.)
2. Nucleotide excision repair (NER): Recognizes bulky, helix-distorting lesions such as pyrimidine dimers.
3. Mismatch repair (MMR): Corrects errors of DNA replication and recombination that result in mispaired (but undamaged) nucleotides.

As noted, a variety of enzymatic processes functions to repair damaged DNA, protecting cells from the potentially mutagenic and lethal effects of chemical agents and oxygen-induced damage. According to Pitot and Dragan (2001), in mammalian systems, there are two basic types of damage responses: repair mechanisms and tolerance mechanisms. The repair mechanisms involve removal of the DNA damage, while the tolerance mechanisms constitute circumvention of the damage without fixing it. A number of enzyme systems exist, facilitating the repair. Four main steps are identified in the repair process in which enzyme systems are involved:

1. Recognition of the lesion by endonucleases.
2. Removal of the damaged portion by exonuclease.
3. Replacement of the damaged section of DNA by polymerases.
4. Rejoining the uninjured parts by ligases.

Although the repair process is quite efficient, errors may occur. Some of the errors may be expressed as visible chromosomal abnormalities, such as breaks, deletions, translocations, ring chromosomes, and sister chromatid exchanges. It is also considered likely that faulty DNA repair may lead to some mutations and cancer.

REVIEW QUESTIONS

1. What is cancer?
2. What are the two most important causes of cancer?
3. What are the three stages of carcinogenesis? Which of these is (are) considered reversible?
4. What is the fundamental principle underlying the many theories about the causes of cancer?
5. What is the main cause of skin cancer?
6. How does short-wave UV radiation affect DNA?
7. Which one is most harmful: UV-A, UV-B, or UV-C?
8. List five metals that can induce cancer.
9. What is metastasis? How does it occur?
10. What are procarcinogens?
11. In what way does the superoxide free radical affect cancer?
12. How does vinyl chloride become a carcinogen?
13. What are nitroso compounds? Explain the way in which nitroso compounds can induce carcinogenesis.
14. Explain how BaP can be converted into its ultimate carcinogen.
15. How may DDE be related to carcinogenesis?
16. Explain the suggested mechanism by which dioxins act as a carcinogen.
17. Explain the mechanism that mammalian systems possess for repairing DNA damage.

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Appendix 1: Ecological Risk Assessment

A1.1 INTRODUCTION

Ecological risk assessment is the process that evaluates the potential adverse effects that human activities have on the plants and animals that make up ecosystems (U.S. Environmental Protection Agency [USEPA] 2004). Ecological risk assessments also consider changes caused by human activities that alter important features of ecological systems, such as lakes, streams, forests, or watersheds. Anthropogenic changes may include, for example, the introduction of a new chemical, such as a pesticide, to a wheat field or alteration of a landscape that results from draining or filling a wetland.

Scientists often assess how much damage certain human actions may have on the plants or animals in the area in question. The risk assessment process provides a way to develop, organize, and present scientific information so that it is relevant to environmental decision making. Ecological risks may be local, such as a hazardous waste site; they may be regional, such as the Pacific Northwest regions of the United States or a certain section of the Mississippi River; or they may be global, such as emission of greenhouse gases, atmospheric transport of particulates, or global warming.

The early 1980s witnessed both the emergence of risk assessment as a regulatory paradigm and the first widespread use of ecological impact assessments to influence regulatory and policy decisions. The use of ecological information for decision making has expanded slowly through the 1980s, as shown by the regulation of diazinon based on its impacts on birds and action taken to tackle acid deposition in lakes (USEPA 2004). In the middle to late 1980s, tools and methods for conducting ecological risk assessments began to be standardized with the publications of several documents by U.S. government agencies, such as the National Research Council and EPA (USEPA 1999, 2004). After nearly two decades of effort and experiences, ecological risk assessment has become widely known as an important management tool to many government officials and environmental scientists.

This chapter presents an introduction to the subject by summarizing several key points from available documents.

A1.2 BASIC COMPONENTS OF RISK ASSESSMENT

It is useful to first become familiar with several important terms commonly used in a risk assessment. These are as follows, with brief definitions:

- *Risk*—the probability of an adverse outcome; a combination of exposure and effects expressed as probability.
- *Stressor*—any physical, chemical, or biological entity that can induce an adverse response on a biological system (synonymous with *agent*).
- *Exposure*—the contact or cooccurrence of a *stressor* with a *receptor*.
- *Hazard*—used in the United States and Canada to refer to intrinsic toxic properties, while internationally it refers to the probability of an adverse outcome.
- *Receptor*—the ecological entity exposed to the *stressor*.
- *Uncertainty*—a lack of confidence in the prediction of a risk assessment that may be due to natural variability in natural processes, errors in conducting an assessment, or incomplete knowledge about a certain specific aspect of exposure.
- *Risk assessor*—an individual or team with the appropriate training or range of expertise necessary to conduct a risk assessment.
- *Risk manager*—an individual, team, or organization who can make decisions or take action concerning alternatives for addressing risk. (In some cases, risk managers may include interested parties or stakeholders.)

A1.3 USE OF ECOLOGICAL RISK ASSESSMENT

The ecological risk assessment process is used to systematically evaluate and organize data, information, assumptions, and uncertainties to help understand and predict the relationships between stressors and ecological effects in a way that is useful for environmental decision making. Assessment may involve physical, chemical, or biological stressors and may include one or many stressors.

As noted, an ecological risk assessment evaluates the potential adverse effects that human activities have on the plants and animals that make up ecosystems. The risk assessment process provides a way to develop, organize, and present scientific information so that it is relevant to environmental decisions. When conducted for a particular place, such as a watershed, the ecological risk assessment process can be used to identify vulnerable and valued resources, prioritize data collection activities, and link human activities with their potential effects. Risk assessments can also provide a focal point for cooperation between local communities and state and federal government agencies.

Ecological risk assessment is one input into environmental management decisions. Other inputs include stakeholder concerns, availability of technical solutions, benefits, equity, costs, legal mandates, and political issues. Risk assessment results provide a basis for comparing different management options, enabling decision makers and the public to make better-informed decisions about the management of ecological resources (USEPA 2004).

Ecological risk assessments can also be used to predict the likelihood of future adverse effects (prospective) or evaluate the likelihood that effects are caused by past exposure to stressors (retrospective). In many cases, both approaches are included in a single risk assessment.

A1.4 IMPORTANCE OF ECOLOGICAL RISK ASSESSMENT

A great deal of research conducted in the field is geared toward the determination of the risk of producing a new product or releasing chemicals, such as a pesticide or an industrial effluent, to ecosystems. As noted, ecological risk assessments are tools that decision makers can use to help them identify and, it is hoped, reduce uncertainty throughout the decision-making process.

Ecosystem assessments follow general concepts, as shown in Figure A1.1, but there is no predetermined set of rules for undertaking an assessment. The general concepts include acknowledgment of stakeholders and their questions, development of situational analyses, identification of limits and trade-offs, development of an understanding of future conditions, and assessment of risk for issues of concern. The primary reason for conducting ecosystem assessments is to provide a framework for decision makers and stakeholders to help them understand and evaluate the consequences of actions concerning regulation or allocation of natural resources within the larger social and ecological context (USEPA 2004).

The endpoints of risk assessment are often set by societal perceptions and values. Although scientific process may be used in collecting information for the assignment of risks, unless a testable hypothesis can be formulated, the scientific method is not being applied. For example, a course of action that has the least ecological risk may

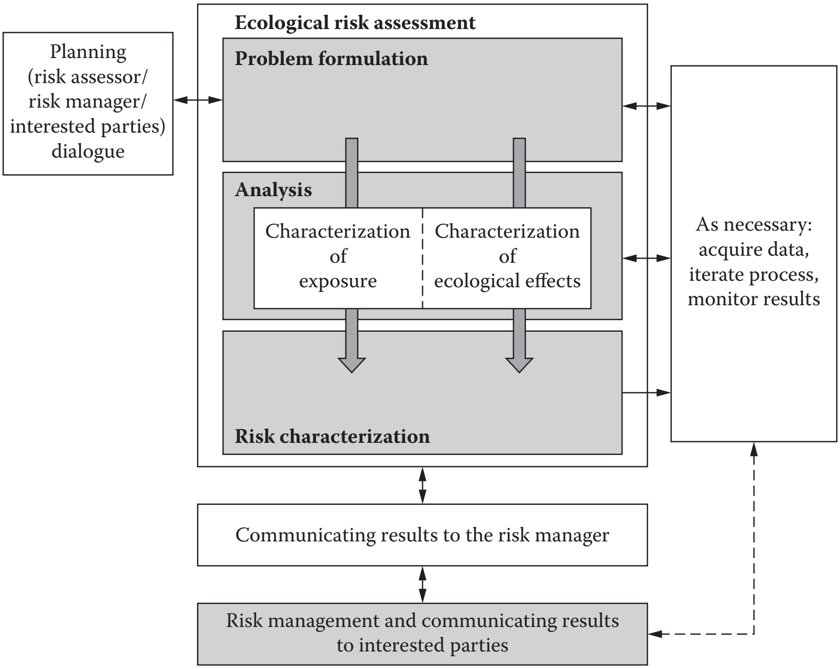
Ecological risk assessment
Problem formulation
Planning (risk assessor/ risk manager/ interested parties) dialogue
Analysis
Characterization of exposure
Characterization of ecological effects
As necessary: acquire data, iterate process, monitor results
Risk characterization
Communicating results to the risk manager
Risk management and communicating results to interested parties

FIGURE A1.1 The framework for ecological risk assessment. (Adapted from U.S. Environmental Protection Agency [USEPA]. Framework for ecological risk assessment. EPA, Washington, DC, 1992.)

be too expensive or not technologically feasible. Therefore, while an ecological risk assessment provides critical information to risk managers, it is only one part of the whole environmental decision-making process.

Environmental toxicology and risk assessment are closely related. Environmental toxicology, as with any branch of science, attempts to answer specific questions. In this case, the question may be primarily focused on how a particular xenobiotic (or xenobiotics) interacts with the components of an ecological system. The background knowledge obtained from the study of environmental toxicology can serve as an important basis for significantly contributing to the process of risk assessment.

A1.5 FRAMEWORKS FOR ECOLOGICAL RISK ASSESSMENT

The ecological risk assessment process is based on two major elements: characterization of effects and characterization of exposure. These elements were proposed over the first decade of the twenty-first century, one of them based on a National Academy of Sciences report detailing risk assessment for federal agencies (National Academy Press 1983).

As shown in Figure A1.1, the framework is composed of three principal elements or phases: problem formulation, analysis, and risk characterization. Problem formulation involves a clear definition of the specific problem under consideration. This phase can ultimately influence the scientific validity and policy related to the risk assessment process. The second phase in the process, analysis, is subdivided into characterization of potential or existing exposure to stressors and characterization of ecological effects. The last step, risk characterization, consists of integration and evaluation of exposure and effects information.

A1.5.1 Problem Formulation

In problem formulation, the purpose for the assessment is stated, the problem is defined, and a plan for analyzing and characterizing risk is determined. The process is made up of several elements: discussion between the risk assessor and risk manager, stressor characteristics, identification of the ecosystem potentially at risk, ecological effects, endpoint selection, conceptual modeling, and input from data acquisition, verification, and monitoring. The initial work in problem formulation includes the integration of available information on sources, stressors, effects, and ecosystem and receptor characteristics. The information obtained contributes to the generation of two products: assessment endpoints and conceptual models. Either product may be generated first (and the order depends on the type of risk assessment), but both are needed to complete an analysis plan, the final product of problem formulation.

The process may be initiated by various causes, for example, as a request for the introduction of a new material into the environment or for the determination of cleanup or land use options for a contaminated site.

A critical aspect of the problem formulation process is the emphasis that is placed on the importance of discussions between the risk assessor and the risk manager, the importance of acquisition of new data, and verification of the risk assessment and monitoring. The discussion between the risk assessor and risk manager of societal goals and scientific reality helps to set the boundaries for the scope of the risk assessment. The interaction between these individuals can help to consolidate the goals into definable components of a risk assessment.

A1.5.2 ANALYSIS

Analysis is directed by the outcome of problem formulation. As indicated, analysis consists of two phases: characterization of exposure and characterization of ecological effects (Figure A1.1). In characterization of exposure, the data resulting from the problem formulation are evaluated to determine how exposure to stressors is likely to occur. The strength and limitations of data concerning exposure, effects, and ecosystem and receptor characteristics are evaluated. As mentioned, exposure is the interaction of stressors with receptors. In the assessment of hazard due to exposure, details of the biological effects of the stressor under examination are assessed. Measures of exposure can include concentrations of contaminants, such as tissue levels of DDT in habitat, or physical changes, such as body weight.

The exposure potential of critical biological components to the material is assessed as part of an exposure characterization. Risk assessment requires qualitative information about the strength of the evidence of the exposure and the nature of the outcomes, as well as quantitative assessment of the exposures, host susceptibility factors, and potential magnitude of the risk, and then a description of the uncertainties in the estimates and conclusions.

Stressor characteristics form an important aspect of the risk assessment process. Stressors can be physical, chemical, or biological in nature. Biological stressors could include the introduction of a new species or the application of a specific fertilizer to farming. Physical stressors may include changes in temperature or geological processes. Examples of chemical stressors may include such materials as pesticides or industrial effluents. Chemical stressors may include intensity, such as dose or concentrations of chemical agents, duration, timing, or frequency of actions.

That step is followed by characterization of ecological effects, that is, determination of the potential and type of ecological effects that can be anticipated. Myriad interactions exist between the stressor and the ecological system, and each should be considered. Examples of interactions include acute and chronic toxicity, bioaccumulation, biodegradation, biotransformation, predator-prey interactions, community resilience, and evolutionary impacts. Available data are analyzed to characterize the nature of potential or actual exposure and the ecological responses under the defined circumstances.

Ecosystems potentially at risk may be more difficult to characterize. Ecosystems include a large number of biotic and abiotic characteristics, and these must be considered in the process. For instance, sediments have both biotic and abiotic components that can dramatically affect contaminant availability. Geographic relationship to nearby systems is another key characteristic influencing species migration and therefore recovery rates from the influence of stressors. In addition, size of the ecosystem is an important variable affecting the number of species and the complexity of the system itself.

A1.5.3 RISK CHARACTERIZATION

The third and final phase of the risk assessment process is risk characterization (Figure A1.1). This involves integration and evaluation of exposure and effects information. The overall process is to combine the ecological effects with the environmental concentrations to provide the likelihood of effects in the presence of the stressor within the system. It is important to point out that a stressor poses no risk to an environment unless it involves exposure. Virtually all materials have some characteristic biological effect; however, unless a sufficient amount of the stressor interacts with a biological system, no effects can occur. Risk is a combination of exposure and resultant effects expressed as a probability. Integrating exposure and effects information leads to an estimation of risk, the likelihood that adverse effects will result from exposure.

Approaches for evaluating exposure and effects include, for example, measuring chemical releases, predicting the environmental fate and effects of chemicals (possibly even before they are manufactured), and testing the effects of these chemicals in a laboratory. Exposure and effects must be considered together because they are both important in assessing risk. When the potential for exposure and effects are low, the risk will be low. When both are high, the risk will be high. Whatever the approach, the goal is to use all available information to characterize exposure and effects and to integrate them into an understanding of ecological risks (Landis and Yu 2004).

The integration of exposure with toxicity needs to be conducted with caution. As noted in the previous chapters, environmental toxicology deals with a variety of effects at different levels of biological organization. A widely used method for estimating risk is the quotient method (Landis and Yu 2004). This method is based on simple division of the expected environmental concentration by the concentration producing an unacceptable effect (i.e., hazard):

$$\text{Quotient} = \frac{\text{Expected environmental concentration}}{\text{Concentration producing an unacceptable effect}}$$

The resultant quotient is generally judged by the following criteria:

Quotient	**Risk**
>1	Potent or high risk
≃1	Potential risk
<1	Low risk

As indicated, because of the complexity of natural systems, risk assessment will include some degree of uncertainty. Although it is possible to reduce some components of uncertainty by collecting additional data, it may only be possible to estimate other components due to their inherent variability (e.g., weather variations). While it is important for risk managers to understand the impact of natural variability and uncertainty on the conclusions of the risk assessment, making a risk management decision does not require the absence of uncertainty. In fact, attempts are normally made to quantify and communicate uncertainty when conducting and reporting ecological risk assessment so that the best decisions can be made given the available knowledge (Society of Environmental Toxicology and Chemistry [SETAC] 1999).

Although analysis and risk characterization are shown as separate phases, some models may combine the analysis of exposure and effects data with the integration of these data that occurs in risk characterization.

A1.6 USEFULNESS OF ECOLOGICAL RISK ASSESSMENT PREDICTIONS

Although there are various sources of uncertainty in ecological risk assessment, it is possible to predict many effects with confidence. Even when uncertainties are high, risk assessments based on proper scientific review and consensus provide the best summary of the state of knowledge.

Ecological risk assessment results are most useful when risk managers clearly communicate the risks and decisions to the public. An ecological risk assessment should

- Summarize results so that the public can understand them
- Distinguish scientific conclusions from policy judgments
- Describe major differences of opinion on scientific conclusions that readers can draw from the data
- Explain major assumptions and uncertainties

Because of the complexity and variability of nature, the initial scoping phase of an ecological risk assessment (problem formulation) is critical for providing a focus for the assessment. However, ecological risk assessments need not be complex or lengthy; they only need to define the risks with the degree of certainty required to support a risk management decision (SETAC 1999).

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Appendix 2: 11th Report on Carcinogens

U.S. Department of Health and Human Services
Public Health Service
National Toxicology Program

Pursuant to Section 301(b) (4) of the Public Health Service Act as Amended by Section 262, PL 95-622

Official Citation: Report on Carcinogens, Eleventh Edition, U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program.

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Chloramphenicol
Chlorendic Acid
Chlorinated Paraffins (C_{12}, 60% Chlorien)
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bis(Chlroethyl) Nitrosourea
Methyl Ether
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4-Chloro-*o*-phenylenediamine
Chloroprene
p-Chloro-*o*-toluidiene and *p*-Chloro-*o*-toluidiene Hydrochloride
Chlorozotocin
Chromium Hexavalent Compounds
C.I. Basic Red 9 Monohydrochloride
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Coal Tars and Coal Tar Pitches
Cobalt Sulfate
Coke Oven Emissions
p-Cresidine
Cupferron
Cyclophosphamide
Cyclosporin A
Dacarbazine
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2,3-Dirbromo-1-propanol
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Diethylstilbestrol
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    Dyes Metabolized to 3,3′-Dimethoxybenzidine
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3,3′-Dimethylbenzidine and Dyes Metabolized to 3,3′-Dimetholbenzidine
    3,3′Dimethoxybenzidine
    Dyes Metabolized to 3,3′Dimethylbenzidine
4-Dimethylaminoazobenzene
3.3′Dimethylbenzidine and Dyes Metabolized to 3,3′-Dimetholbenzidine
    3.3′-Dimethylbenzidine
    Dyes Metabolized to 3,3′Dimethylbenzine
Dimethylcarbamoyl Chloride
1,1′-Dimethylhydrazine
Dimethyl Sulfate
Dimethylvinyl Chloride
1.4-Dioxane
Disperse Blue 1
Epichlorhydrin
Erionite
Estrogens, Steroidal
Ethylene Oxide
Ethylene Thiourea
di(2-Ethylhexyl) Phthalate
Ethyl Methanesulfonate
Formaldehyde (Gas)
Furan
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Glycidol
Hepatitis B
Hepatitis C

- Heterocyclic Amines
  - 2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MEIQ)
  - 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinozaline (MEIQx)
  - 2-Amino-3methylimidazo[4,5-*f*]quinoline (IQ)
  - 2-Amino-1-emthyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)
- Hexachlorobenzene
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- Hydrazobenzene
- Ionizing Radiation
  - X-Radiation and Gamma Radiation
  - Neutrons
  - Radon
  - Thorium Dioxide
- Iron Dextran Complex
- Isoprene
- Kepone® (Chlordecone)
- Lead and Lead Compounds
- Lindane and Other Hexachlorocyclohexane Isomers
- Melphalan
- Methoxsalen with Ultraviolet A Therapy (PUVA)
- 2-Methylaziridine (Propyleneimine)
- 4,4′Methylenebis (2-chloroaniline)
- 4,4′Methylenebis(*N*,*N*-dimethyl)benzenamide
- Metronidazole
- Michler′s Ketone (4,4′-(Dimethylamino)benzophenone)
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- Mustard Gas
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- Nickel Compounds and Metallic Nickel
  - Nickel Compounds
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- *o*-Nitroanisole
- Nitroarene (selected)
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  - 1,8-Dinitropyrene
  - 6-Nitrochrysene
  - 1-Nitropyrene
  - 4-Nitropyrene
- Nitrobenzene
- Nitrofen (2,4-Dichloropheyl-*p*-nitrophenyl Ether)

Nitrogen Mustard Hydrochloride
Nitromethane
2-Nitropropane
N-Nitrosodi-n-butylamine
N-Nitrosodiethanolamine
N-Nitrosodiethylamine
N-Nitrosodimethylamine
N-Nitrosodi-*n*-prophylamine
N-Nitroso-*N*-ethylurea
N-Nitrosomethylvinyl amine
N-Nitrosomorpholine
N-Nitrosonornicotine
N-Nitrosopiperidine
N-Nitrososarcosine
Norethisterone
Ochratoxin A
4,4′-Oxdianiline
Oxymetholone
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    Analgesic Mixtures Containing Phenacetin
Phenazopyridine Hydrochloride
Phenolphthalein
Phenoxybenzamine Hydrochloride
Phenytoin
Polybrominated Biphenyls (PBBs)
Polychlorinated Biphenyls (PCBs)
Polycyclic Aromatic Hydrocarbons, 15 listings
    Benzo[*a*]anthacene
    Benzo[*b*]fluoranthene
    Benzo[*j*]fluorantheene
    Benzo[*k*]fluoranthene
    Benzo[*a*]pyrene
    Dibenz[*a*,*h*]acridine
    Dibenz[*a,j*]acridine
    Dibenz[*a,h*]anthracene
    7H-Dibenzo[*c,g*]carbazole
    Dibenzo[*a,e*]pyrene
    Dibenzo[*a,h*]pyrene
    Dibenzo[*a,i*]pyrene
    Dibenzo[*a,l*]pyrene
    Indenol[1,2,3-*cd*]pyrene
    5-Methylchrysene
Procarbazine Hydrochloride
Progesterone
1,3-Propane Sultone

β-Propiolactone
Propylene Oxide
Propylthiouracil
Reserpine
Safrole
Selenium Sulfide
Silica, Crystalline (Respirable Size)
Soots
Streptozotocin
Strong Inorganic Acid Mists Containing Sulfuric Acid
Styrene-7,8-oxide
Sulfallate
Tamoxifen
2,3,7,8-Tetrachlorodibenzo-pdioxin (TCKK); "Dioxin"
Tetrachloroethylene (Perchloroethylene)
Tetrafluoroethylene
Tetranitromethane
Thioacetamide
4,4-Thiodianiline
Thiotepa
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Tobacco Related Exposures
    Environmental Tobacco Smoke
    Smokeless Tobacco
    Tobacco Smoking
Toluene Diisocyanate
o-Toluidine and *0*-Toluidine Hydrochloride
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Trichloroethylene
2,4,6-Trichlorphenol
1,2,3-Trichloropropane
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    Ultraviolet B Radiation
    Ultraviolet C Radiation
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Vinyl bromide
Vinyl Chloride
4-Vinyl-1-cyclohexene Diepoxide
Vinyl Fluoride
Wood Dust

Appendix 3: List of Cigarette Smoke Carcinogens*

According to the U.S. Department of Health and Human Services,[1] the following are known human carcinogens found in cigarette smoke:

Chemical	**Amount (per cigarette)**
Acetaldehyde	980 micrograms to 1.37 milligrams
Acrylonitrile	formerly 1 to 2 milligrams. This product was used as a fumigant in tobacco. Its use has since been discontinued.
4-Aminobiphenyl	0.2 to 23 nanograms per cigarette
o-Anisidine hydrochloride	unknown
Arsenic	unknown
Benzene	5.9 to 75 micrograms
Beryllium	0.5 nanograms
1,3-Butadiene	152 to 400 micrograms
Cadmium	1.7 micrograms
1,1-Dimethylhydrazine	unknown
Ethylene oxide	unknown
Formaldehyde	unknown
Furan	unknown
Heterocyclic amines	unknown
Hydrazine	32 micrograms
Isoprene	:3.1 milligrams
Lead	unknown
2-Naphthylamine	1.5 to 35 nanograms
Nitromethane	unknown
N-Nitrosodi-*n*-butylamine	3 nanograms
N-Nitrosodiethanolamine	24 to 36 nanograms
N-Nitrosodiethylamine	up to 8.3 nanograms
N-Nitrosodimethylamine	5.7 to 43 nanograms
4-(*N*-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone	up to 4.2 micrograms
N-Nitrosonornicotine	14 micrograms
N-Nitrosopiperidine	unknown
N-Nitrosopyrrolidine	113 nanograms
N-Nitrososarcosine	22 to 460 nanograms

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* From Wikipedia, the free encyclopedia.

Chemical	Amount (per cigarette)
Polonium-210	variable, depending on soil and fertilizer used to grow tobacco[2]
Polycyclic aromatic hydrocarbons	28 to 100 milligrams
o-Toluidine	32 nanograms
Vinyl chloride	5.6 to 27 nanograms

See also

- List of additives in cigarettes
- Health effects of tobacco smoking

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Appendix 4: Polychlorinated Biphenyl (PCB) Nomenclature

BZ&IUPAC #	IUPAC Name	CASRN
1	2-Chlorobiphenyl	2051-60-7
2	3-Chlorobiphenyl	2051-61-8
3	4-Chlorobiphenyl	2051-62-9
4	2,2′-Dichlorobiphenyl	13029-08-8
5	2,3-Dichlorobiphenyl	16605-91-7
6	2,3′-Dichlorobiphenyl	25569-80-6
7	2,4-Dichlorobiphenyl	33284-50-3
8	2,4′-Dichlorobiphenyl	34883-43-7
9	2,5-Dichlorobiphenyl	34883-39-1
10	2,6-Dichlorobiphenyl	33146-45-1
11	3,3′-Dichlorobiphenyl	2050-67-1
12	3,4-Dichlorobiphenyl	2974-92-7
13	3,4′-Dichlorobiphenyl	2974-90-5
14	3,5-Dichlorobiphenyl	34883-41-5
15	4,4′-Dichlorobiphenyl	2050-68-2
16	2,2′-3-Trichlorobiphenyl	38444-78-9
17	2,2′,4-Trichlorobiphenyl	37680-66-3
18	2,2′,5-Trichlorobiphenyl	37680-65-2
19	2,2′,6-Trichlorobiphenyl	38444-73-4
20	2,3,3′-Trichlorobiphenyl	38444-84-7
21	2,3,4-Trichlorobiphenyl	55702-46-0
22	2,3,4′-Trichlorobiphenyl	38444-85-8
23	2,3,5-Trichlorobiphenyl	55720-44-0
24	2,3,6-Trichlorobiphenyl	55702-45-9
25	2,3′,4-Trichlorobiphenyl	55712-37-3
26	2,3′,5-Trichlorobiphenyl	38444-81-4
27	2,3′,6-Trichlorobiphenyl	38444-76-7
28	2,4,4′-Trichlorobiphenyl	7012-37-5
29	2,4,5-Trichlorobiphenyl	15862-07-4
30	2,4,6-Trichlorobiphenyl	35693-92-6
31	2,4′,5-Trichlorobiphenyl	16606-02-3
32	2,4′,6-Trichlorobiphenyl	38444-77-8
33	2,3′,4′-Trichlorobiphenyl	38444-86-9
34	2,3′,5′-Trichlorobiphenyl	37680-68-5
35	3,3′,4-Trichlorobiphenyl	37680-69-6
36	3,3′,5-Trichlorobiphenyl	38444-87-0

BZ&IUPAC #	IUPAC Name	CASRN
37	3,4,4′-Trichlorobiphenyl	38444-90-5
38	3,4,5-Trichlorobiphenyl	53555-66-1
39	3,4′,5-Trichlorobiphenyl	38444-88-1
40	2,2′,3,3′-Tetrachlorobiphenyl	38444-93-8
41	2,2′,3,4-Tetrachlorobiphenyl	52663-59-9
42	2,2′,3,4′-Tetrachlorobiphenyl	36559-22-5
43	2,2′,3,5-Tetrachlorobiphenyl	70362-46-8
44	2,2′,3,5′-Tetrachlorobiphenyl	41464-39-5
45	2,2′,3,6-Tetrachlorobiphenyl	70362-45-7
46	2,2′,3,6′-Tetrachlorobiphenyl	41464-47-5
47	2,2′,4,4′-Tetrachlorobiphenyl	2437-79-8
48	2,2′,4,5-Tetrachlorobiphenyl	70362-47-9
49	2,2′,4,5′-Tetrachlorobiphenyl	41464-40-8
50	2,2′,4,6-Tetrachlorobiphenyl	62796-65-0
51	2,2′,4,6′-Tetrachlorobiphenyl	68194-04-7
52	2,2′,5,5′-Tetrachlorobiphenyl	35693-99-3
53	2,2′,5,6′-Tetrachlorobiphenyl	41464-41-9
54	2,2′,6,6′-Tetrachlorobiphenyl	15968-05-5
55	2,3,3′,4-Tetrachlorobiphenyl	74338-24-2
56	2,3,3′,4′-Tetrachlorobiphenyl	41464-43-1
57	2,3,3′,5-Tetrachlorobiphenyl	70424-67-8
58	2,3,3′,5′-Tetrachlorobiphenyl	41464-49-7
59	2,3,3′,6-Tetrachlorobiphenyl	74472-33-6
60	2,3,4,4′-Tetrachlorobiphenyl	33025-41-1
61	2,3,4,5-Tetrachlorobiphenyl	33284-53-6
62	2,3,4,6-Tetrachlorobiphenyl	54230-22-7
63	2,3,4′,5-Tetrachlorobiphenyl	74472-34-7
64	2,3,4′,6-Tetrachlorobiphenyl	52663-58-8
65	2,3,5,6-Tetrachlorobiphenyl	33284-54-7
66	2,3′,4,4′-Tetrachlorobiphenyl	32598-10-0
67	2,3′,4,4′-Tetrachlorobiphenyl	73575-53-8
68	2,3′,4,5′-Tetrachlorobiphenyl	73575-52-7
69	2,3′,4,6-Tetrachlorobiphenyl	60233-24-1
70	2,3′,4′,5-Tetrachlorobiphenyl	32598-11-1
71	2,3′,4′,6-Tetrachlorobiphenyl	41464-46-4
72	2,3′,5,5′-Tetrachlorobiphenyl	41464-42-0
73	2,3′,5′,6-Tetrachlorobiphenyl	74338-23-1
74	2,4,4′,5-Tetrachlorobiphenyl	32690-93-0
75	2,4,4′,6-Tetrachlorobiphenyl	32598-12-2
76	2,3′,4′,5′-Tetrachlorobiphenyl	70362-48-0
77	3,3′,4,4′-Tetrachlorobiphenyl	32598-13-3
78	3,3′,4,5-Tetrachlorobiphenyl	70362-49-1
79	3,3′,4,5′-Tetrachlorobiphenyl	41464-48-6
80	3,3′,5,4′-Tetrachlorobiphenyl	33284-52-5

BZ&IUPAC #	IUPAC Name	CASRN
81	3,4,4′,5-Tetrachlorobiphenyl	70362-50-4
82	2,2′,3,3′,4-Pentachlorobiphenyl	52663-62-4
83	2,2′,3,3′,5-Pentachlorobiphenyl	60145-20-2
84	2,2′,3,3′,6-Pentachlorobiphenyl	52663-60-2
85	2,2′,3,4,4′-Pentachlorobiphenyl	65510-45-4
86	2,2′,3,4,5-Pentachlorobiphenyl	55312-69-1
87	2,2′,3,4,5′-Pentachlorobiphenyl	38380-02-8
88	2,2′,3,4,6-Pentachlorobiphenyl	55215-17-3
89	2,2′,3,4,6′-Pentachlorobiphenyl	73575-57-2
90	2,2′,3,4′,5-Pentachlorobiphenyl	69194-07-0
91	2,2′,3,4′,6-Pentachlorobiphenyl	68194-05-8
92	2,2′,3,5,5′-Pentachlorobiphenyl	52663-61-3
93	2,2′,3,5,6-Pentachlorobiphenyl	73575-56-1
94	2,2′,3,5,6′-Pentachlorobiphenyl	73575-55-0
95	2,2′,3,5′,6-Pentachlorobiphenyl	38379-99-6
96	2,2′,3,6,6′-Pentachlorobiphenyl	73575-54-9
97	2,2′,3,4′,5′-Pentachlorobiphenyl	41464-51-1
98	2,2′,3,4′,6′-Pentachlorobiphenyl	60233-25-2
99	2,2′,4,4′,5-Pentachlorobiphenyl	38380-01-7
100	2,2′,4,4′,6-Pentachlorobiphenyl	39485-83-1
101	2,2′,4,5,5′-Pentachlorobiphenyl	37680-73-2
102	2,2′,4,5,6′-Pentachlorobiphenyl	68194-06-9
103	2,2′,4,5′,6-Pentachlorobiphenyl	60145-21-3
104	2,2′,4,6,6′-Pentachlorobiphenyl	56558-16-8
105	2,3,3′4,4′-Pentachlorobiphenyl	32598-14-4
106	2,3,3′4,5-Pentachlorobiphenyl	70424-69-0
107	2,3,3′,4′,5-Pentachlorobiphenyl	70424-68-9
108	2,3,3′,4,5′-Pentachlorobiphenyl	70362-41-3
109	2,3,3′,4,6-Pentachlorobiphenyl	74427-35-8
110	2,3,3′,4′,6-Pentachlorobiphenyl	38380-03-9
111	2,3,3′,5,5′-Pentachlorobiphenyl	39635-32-0
112	2,3,3′,5,6-Pentachlorobiphenyl	74427-36-9
113	2,3,3′,5′,6-Pentachlorobiphenyl	68194-10-5
114	2,3,4,4′,5-Pentachlorobiphenyl	74427-37-0
115	2,3,4,4′,6-Pentachlorobiphenyl	74427-38-1
116	2,3,4,5,6-Pentachlorobiphenyl	18259-05-7
117	2,3,4′,5,6-Pentachlorobiphenyl	68194-11-6
118	2,3′,4,4′,5-Pentachlorobiphenyl	31508-00-6
119	2,3′,4,4′,6-Pentachlorobiphenyl	56558-17-9
120	2,3′,4,5,5′-Pentachlorobiphenyl	68194-12-7
121	2,3′,4,5′,6-Pentachlorobiphenyl	56558-18-0
122	2,3,3′,4′,5′-Pentachlorobiphenyl	76842-07-4
123	2,3′,4,4′,5′-Pentachlorobiphenyl	65510-44-3
124	2,3′,4′,5,5′-Pentachlorobiphenyl	70424-70-3

BZ&IUPAC #	IUPAC Name	CASRN
125	2,3′,4′,5′,6-Pentachlorobiphenyl	74427-39-2
126	3,3′,4,4′,5-Pentachlorobiphenyl	57465-28-8
127	3,3′,4,5,5′-Pentachlorobiphenyl	39635-33-1
128	2,2′,3,3′,4,4′-Hexachlorobiphenyl	38380-07-3
129	2,2′,3,3′,4,5-Hexachlorobiphenyl	55215-18-4
130	2,2′,3,3′,4,5′-Hexachlorobiphenyl	52663-66-8
131	2,2′,3,3′,4,6-Hexachlorobiphenyl	61798-70-7
132	2,2′,3,3′,4,6′-Hexachlorobiphenyl	38380-05-1
133	2,2′,3,3′,5,5′-Hexachlorobiphenyl	35694-04-3
134	2,2′,3,3′,5,6-Hexachlorobiphenyl	52704-70-8
135	2,2′,3,3′,5,6′-Hexachlorobiphenyl	52744-13-5
136	2,2′,3,3′,6,6′-Hexachlorobiphenyl	38411-22-2
137	2,2′,3,4,4′,5-Hexachlorobiphenyl	35694-06-5
138	2,2′,3,4,4′,5′-Hexachlorobiphenyl	35065-28-2
139	2,2′,3,4,4′,6-Hexachlorobiphenyl	56030-56-9
140	2,2′,3,4,4′,6′-Hexachlorobiphenyl	59291-64-4
141	2,2′,3,4,5,5′-Hexachlorobiphenyl	52712-04-6
142	2,2′,3,4,5,6-Hexachlorobiphenyl	41411-61-4
143	2,2′,3,4,5,6′-Hexachlorobiphenyl	68194-15-0
144	2,2′,3,4,5′,6-Hexachlorobiphenyl	68194-14-9
145	2,2′,3,4,6,6′-Hexachlorobiphenyl	74472-40-5
146	2,2′,3,4′,5,5′-Hexachlorobiphenyl	51908-16-8
147	2,2′,3,4′,5,6-Hexachlorobiphenyl	68194-13-8
148	2,2′,3,4′,5,6′-Hexachlorobiphenyl	74472-41-6
149	2,2′,3,4′,5′,6-Hexachlorobiphenyl	38380-04-0
150	2,2′,3,4′,6,6′-Hexachlorobiphenyl	68194-08-1
151	2,2′,3,5,5′,6-Hexachlorobiphenyl	52663-63-5
152	2,2′,3,5,6,6′-Hexachlorobiphenyl	68194-09-2
153	2,2′,4,4′,5,5′-Hexachlorobiphenyl	35065-27-1
154	2,2′,4,4′,5,6′-Hexachlorobiphenyl	60145-22-4
155	2,2′,4,4′,6,6′-Hexachlorobiphenyl	33979-03-2
156	2,3,3′,4,4′,5-Hexachlorobiphenyl	38380-08-4
157	2,3,3′,4,4′,5′-Hexachlorobiphenyl	69782-90-7
158	2,3,3′,4,4′,6-Hexachlorobiphenyl	74472-42-7
159	2,3,3′,4,5,5′-Hexachlorobiphenyl	39635-35-3
160	2,3,3′,4,5,6-Hexachlorobiphenyl	41411-62-5
161	2,3,3′,4,5′,6-Hexachlorobiphenyl	74472-43-8
162	2,3,3′,4,5,5′-Hexachlorobiphenyl	39635-34-2
163	2,3,3′,4′,5,6-Hexachlorobiphenyl	74472-44-9
164	2,3,3′,4′,5′,6-Hexachlorobiphenyl	74472-45-0
165	2,3,3′,5,5′,6-Hexachlorobiphenyl	74472-46-1
166	2,3,4,4′,5,6-Hexachlorobiphenyl	41411-63-6
167	2,3,4,4′,5,5′-Hexachlorobiphenyl	52663-72-6
168	2,3,4,4′,5′,6-Hexachlorobiphenyl	59291-65-5

BZ&IUPAC #	IUPAC Name	CASRN
169	3,3′,4,4′,5,5′-Hexachlorobiphenyl	32774-16-6
170	2,2′,3,3′,4,4′,5-Hexachlorobiphenyl	35065-30-6
171	2,2′,3,3′,4,4′,6-Hexachlorobiphenyl	52663-71-5
172	2,2′,3,3′,4,5,5′-Hexachlorobiphenyl	52663-74-8
173	2,2′,3,3′,4,5,6-Hexachlorobiphenyl	68194-16-1
174	2,2′,3,3′,4,5,6′-Hexachlorobiphenyl	38411-25-5
175	2,2′,3,3′,4,5′,6-Hexachlorobiphenyl	40186-70-7
176	2,2′,3,3′,4,6,6′-Hexachlorobiphenyl	52663-65-7
177	2,2′,3,3′,4,5′,6′-Hexachlorobiphenyl	52663-70-4
178	2,2′,3,3′,5,5′,6-Hexachlorobiphenyl	52663-67-9
179	2,2′,3,3′,5,6,6′-Heptachlorobiphenyl	52663-64-6
180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	35065-29-3
181	2,2′,3,4,4′,5,6-Heptachlorobiphenyl	74472-47-2
182	2,2′,3,4,4′,5,6′-Heptachlorobiphenyl	60145-23-5
183	2,2′,3,4,4′,5′,6-Heptachlorobiphenyl	52663-69-1
184	2,2′,3,4,4′,6,6′-Heptachlorobiphenyl	74472-48-3
185	2,2′,3,4,5,5′,6-Heptachlorobiphenyl	52712-05-7
186	2,2′,3,4,5,6,6′-Heptachlorobiphenyl	74472-49-4
187	2,2′,3,4′,5,5′,6′-Heptachlorobiphenyl	52663-68-0
188	2,2′,3,4′,5,6,6′-Heptachlorobiphenyl	74487-85-7
189	2,3,3′,4,4′,5,5′-Heptachlorobiphenyl	39635-31-9
190	2,3,3′,4,4′,5,6-Heptachlorobiphenyl	41411-64-7
191	2,3,3′,4,4′,5′,6-Heptachlorobiphenyl	74472-50-7
192	2,3,3′,4,5,5′,6-Heptachlorobiphenyl	74472-51-8
193	2,3,3′,4,5,5′,6-Heptachlorobiphenyl	69782-91-8
194	2,2′,3,3′,4,4′,5,5′-Octachlorobiphenyl	35694-08-7
195	2,2′,3,3′,4,4′,5,6-Octachlorobiphenyl	52663-78-2
196	2,2′,3,3′,4,4′,5,6′-Octachlorobiphenyl	42740-50-1
197	2,2′,3,3′,4,4′,6,6′-Octachlorobiphenyl	33091-17-7
198	2,2′,3,3′,4,5,5′,6-Octachlorobiphenyl	68194-17-2
199	2,2′,3,3′,4,5,5′,6′-Octachlorobiphenyl	52663-75-9
200	2,2′,3,3′,4,5,6,6′-Octachlorobiphenyl	52663-73-7
201	2,2′,3,3′,4,5′,6,6′-Octachlorobiphenyl	40186-71-8
202	2,2′,3,3′,5,5′,6,6′-Octachlorobiphenyl	2136-99-4
203	2,2′,3,4,4′,5,5′,6-Octachlorobiphenyl	74472-52-9
204	2,2′,3,4,4′,5,6,6′-Octachlorobiphenyl	74472-52-9
205	2,3,3′,4,4′,5,5′,6-Octachlorobiphenyl	74472-53-0
206	2,2′,3,3′,4,4′,5,5′,6-Octachlorobiphenyl	40186-72-9
207	2,2′,3,3′,4,4′,5,6,6′-Octachlorobiphenyl	52663-79-3
208	2,2′,3,3′,4,5,5′,6,6′-Octachlorobiphenyl	52663-77-1
209	Decachlorobiphenyl	2051-24-3

HOMOLOGS

BZ&IUPAC#	IUPAC Name	CASRN	BZ&IUPAC#	IUPAC Name	CASRN
	Monochlorobiphenyl	27323-18-8		Hexachlorobiphenyl	26601-64-9
	Dichlorobiphenyl	25512-42-9		Hexachlorobiphenyl	28655-71-2
	Trichlorobiphenyl	25323-68-6		Octachlorobiphenyl	55722-26-4
	Tetrachlorobiphenyl	26914-33-0		Nonachlorobiphenyl	53742-07-7
	Pentachlorobiphenyl	25429-29-2			

MIXTURES

BZ&IUPAC#	IUPAC Name	CASRN	BZ&IUPAC#	IUPAC Name	CASRN
	Aroclor 1016	12674-11-2		Aroclor 1248	12672-29-6
	Aroclor 1210	147601-87-4		Aroclor 1250	165245-51-2
	Aroclor 1216	151820-27-8		Aroclor 1252	89577-78-6
	Aroclor 1221	11104-28-2		Aroclor 1254	11097-69-1
	Aroclor 1231	37234-40-5		Aroclor 1260	11096-82-5
	Aroclor 1232	11141-16-5		Aroclor 1262	37324-23-5
	Aroclor 1240	71328-89-7		Aroclor 1268	11100-14-4
	Aroclor 1242	53469-21-9		Aroclor (unspecified)	12767-79-2

Note: BZ = Ballschmiter-Zellsystem; IUPAC = International Union of Pure and Applied Chemists.

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